Volume 4 September 1952 Number 3

PHARMACOLOGICAL REVIEWS

A quarterly publication in pharmacology, experimental therapeutics, and chemotherapy published for the AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INC., under the supervision of the Board of Publication Trustees: M. H. Seevers, *Chairman* McKeen Cattell, L. S. Goodman, H. B. van Dyke and Arnold D. Welch.

The Editorial supervision of Pharmacological Reviews is shared jointly with the BRITISH PHARMACOLOGICAL SOCIETY and the SCANDINAVIAN PHARMACOLOGICAL SOCIETY.

EDITORIAL BOARD

L. S. Goodman—Salt Lake City, *Chairman*

J. H. Burn—Oxford
J. H. Gaddum—Edinburgh
Thomas C. Butler—Chapel Hill
Alfred Gilman—New York, N. Y.
Harry Eagle—Washington, D. C.
Erik Jacobsen—Copenhagen
U. S. v. Euler—Stockholm
Otto Krayer—Boston

Miles Weatherall—London

CONTENTS

The Methonium Compounds *by* W. D. M. Paton and Eleanor J. Zaimis.... 219

Antidiuretic Substances *by* Mary Pickford.............................. 254

Quantitative Aspects of Iodine Metabolism in Man *by* Douglas S. Riggs.... 284

Published by

THE WILLIAMS & WILKINS COMPANY

BALTIMORE 2, MD., U. S. A.

PHARMACOLOGICAL REVIEWS

Issued four times a year: March, June, September, and December. One volume of approximately 480 pages per year, subscription price is $6.00 per volume. For countries outside the postal union add $.50 per volume. Single copy price $2.00. Correspondence regarding subscriptions should be addressed to the publishers, or their agents.

Claims for copies lost in the mails must be received within 30 days (domestic). Changes of address must be received within two weeks of the date of issue.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received a pro-rata refund will be made to the subscriber.

To avoid a break in your series, subscriptions should be renewed promptly. The publishers cannot guarantee to supply back issues on belated renewals.

Journal is not responsible for loss of manuscript in the mail. Authors should retain a copy.

THE WILLIAMS & WILKINS COMPANY
Publishers of Scientific Books and Periodicals
BALTIMORE 2, MARYLAND, U.S.A.

Entered as second-class matter at the post office at Baltimore 1, Md.
Copyright 1952 by the Williams and Wilkins Company
Made in the United States of America

THE METHONIUM COMPOUNDS

W. D. M. PATON[*] AND ELEANOR J. ZAIMIS

National Institute for Medical Research, Mill Hill, and School of Pharmacy, London

I. Introduction 219
II. Physical and chemical properties 221
III. Estimation 221
IV. Excretion, absorption and distribution 222
V. Neuromuscular action of methonium salts 224
  (1) Modes of neuromuscular block 224
  (2) Characteristics of competitive neuromuscular block 225
  (3) Characteristics of neuromuscular block due to endplate depolarisation 226
    (a) Cat muscle: depolarization of the motor endplate 227
    (b) Avian muscle 229
    (c) Frog muscle 229
  (4) Relation of mode of action to differences between decamethonium and *d*-tubocurarine 229
  (5) Neuromuscular block of intermediate character 231
  (6) Variation of potency of decamethonium with species 232
  (7) Action of decamethonium in man 232
  (8) Relation to other neuromuscular blocking agents 233
VI. Ganglionic blocking actions of methonium salts 234
  (1) Site and mode of action 235
  (2) Cardiovascular actions 236
  (3) Gastrointestinal effects 236
  (4) Other ganglionic blocking actions 237
  (5) Other actions of hexamethonium of uncertain mechanism 237
  (6) Factors influencing the response to hexamethonium 238
    (a) Background tone 238
    (b) Variations in sensitivity 238
    (c) The possibility of maximal block 239
    (d) Intensity and duration of activity 239
    (e) Compensatory mechanisms 239
  (7) Hypertension 239
  (8) Reduction of bleeding during surgical operations 241
  (9) Actions on the human alimentary tract 243
  (10) Comparison of tetra-, penta- and hexamethonium 243
  (11) Other actions of hexamethonium in man 244
  (12) Toxicity 245
VII. Other members of the methonium series and other actions 245
VIII. Specificity, potency, site of action and chemical structure 245
IX. Conclusions 247

I. Introduction

Within the last twenty years decisive evidence has accumulated that transmission of excitation from nerve to skeletal muscle or across the ganglionic synapse is achieved by means of chemical mediation. It was from the study of the actions of drugs such as nicotine, curare and the choline esters, most of them quaternary in nature, that the chemical theory of transmission grew. At first

---

[*] Present address: University College Hospital Medical School, London W.C.1.

the interest was chiefly in the clash with the electrical theory of transmission. But at the same time as this issue was being settled, a much better theoretical basis for the understanding of the properties of quaternary salts in general was being gained. The acceptance of the theory and the use of the techniques developed by Dale and his colleagues clarified at once the actions of well-known drugs such as eserine. Moreover it stimulated the search for new drugs with specific activities in preserving, mimicking or antagonizing the natural transmitter. It would be true to say that all discussion of drugs causing neuromuscular or ganglionic block now rests on the theory of chemical transmission, and that a large part of the experimental work on such drugs consists of a recapitulation, in one form or another, of the original experiments.

Essential to the study of such substances also were the isolation and determination of structure of *d*-tubocurarine by Harold King, the success of Bovet and his colleagues in constructing curarizing compounds synthetically, and the revival of interest in autonomic function and in ganglion blocking agents initiated by Acheson, Moe, Hoobler and Lyons. All these developments provided the background without which the recognition of the activities of the methonium salts and the analysis of these actions would have been slow and difficult. Important as this background is, however, it cannot be discussed in detail here, and the reader must be referred to the numerous reviews on the subject.

A few members of the polymethylene bistrimethyl-ammonium series, whose general formula is

$$(CH_3)_3\overset{+}{N}\cdot(CH_2)_n\cdot\overset{+}{N}(CH_3)_3$$

were synthesised many years ago, between 1895 and 1926, as diodides or dipicrates (namely, the derivatives with n = 2, 4, 5, 6, 7 and 10; for references, see 185). The octamethylene compound, whose properties initiated our investigations, was first prepared by Harold King in recent years. A complete series of diodides, ranging from the dimethylene up to the tridecamethylene derivative, was synthesised by Zaimis in 1948 (135). At the same time, Barlow and Ing (15, 16) independently prepared the dibromides, omitting the hexamethylene member which was later described by Balaban, Levy and Wilde (9). Paton and Zaimis (135–142) confined themselves from the start to this particular series of salts, analysing in detail those compounds whose peculiarities and potency had drawn their attention. Barlow and Ing, at the same time as synthesizing the dibromides of the above series, prepared dibromides of the bistriethylammonium, bis-strychninium, bisquinolinium and bisphenyldimethylammonium series, concerning themselves particularly with the "curare-like" activity of these salts in relation to the inter-quaternary distance, rather than with the general pharmacology of the active members.

The name of "methonium" compounds for the members of the polymethylene bistrimethylammonium series has been approved by the British Pharmacopoeia Commission, and it is under this name, preceded by the appropriate numerical prefix, that they are now known.

The pharmacological studies have shown this series to contain two very specific compounds: decamethonium, imitating acetylcholine very closely at the neuromuscular junction of certain species; and hexamethonium, specifically antagonising acetylcholine at the ganglionic synapse.

II. Physical and Chemical Properties

The methonium di-iodides are white crystalline solids, not markedly hygroscopic, very soluble in water, and form neutral solutions. The solubility of the octadecane derivative, the only higher member so far synthesized, is lower than that of the other members of the series, as would be expected. The di-iodides are stable in aqueous solutions, and can be sterilized by boiling or autoclaving. They may be boiled with acid or alkali without destruction.

The dibromides have the same properties as the di-iodides except for being both hydrated and hygroscopic. The dichlorides are still more hygroscopic than the bromides, their lower members often being difficult to dispense in the solid state (134). The uptake of moisture depends in part on the method of preparation. The bitartrate and methosulphate salts have also been prepared (8, 12).

The lower members of the methonium series have negligible surface activity; with dodecamethonium and tridecamethonium a distinct depression of the surface-tension of water is observed which becomes substantial with the octadecane derivative.

The methonium salts form insoluble salts with ammonium reineckate, and their isolation from body fluids and photoelectric determination are based on this property (185). Sparingly soluble salts of hexamethonium are formed with 2:2′-dihydroxy-1:1′-dinaphthyl methane-3:3′-dicarboxylic acid ("embonic acid") and with its next lower homologue ("bis-oxynaphthoic acid"), and these have proved useful in the isolation of hexamethonium from mixtures (13).

All the soluble salts have been successfully given intravenously, intramuscularly, subcutaneously and orally. They are non-irritant unless given in strongly hypertonic solution (*e.g.*, 30%). The incorporation of hexamethonium in a delay-medium of polyvinyl-pyrrolidone (20%) has been used, but absorption from the site of injection is only slightly prolonged (118).

III. Estimation

Colorimetric and biological methods have been described for the detection of methonium compounds in urine, faeces and blood.

Zaimis (185) has described a photoelectric method for the determination of methonium ions, after their precipitation as reineckates. The method may be applied to urine or faeces. At least 10 mg. of methonium salt is required for an adequate estimation. The technique is therefore not sensitive enough to analyse the methonium content of the blood. The reaction used for the detection of small amounts of tetraethylammonium does not proceed for the methonium series.

For detecting low dilutions of hexamethonium, a biological method must be used. Two procedures have proved successful (129): (a) use of the antagonism of hexamethonium to acetylcholine contracture of the frog's rectus; this permits estimations with limits of error of ±10% or better, and requires about 1 mg. of hexamethonium ion; (b) use of the blocking action of hexamethonium injected close arterially into the continuously excited superior cervical ganglion of the cat, with ganglionic transmission recorded by the contraction of the nictitating membrane. Concentrations of 0.5 μg./cc. are detectable, in a volume of 0.3– 0.5 cc.; the method is subject to the usual errors of biological assay, but estimates within 0.5 μg./cc. are readily obtained.

For detecting small amounts of decamethonium, such as appear in the urine of patients receiving the drug, the response of avian muscle can be used (187). An intravenous injection of a dose of 30 μg./kg. of decamethonium produces a retraction of the head and extension of the limbs; a typical response can be obtained with 2–3 μg. decamethonium in a chick a few days old. The test may be made with urine directly, if a volume not greater than 0.5 cc. is injected rather slowly. The contracture appears during the injection and a convenient endpoint is when the chick is again able to stand normally. The decamethonium content is determined from the duration of the contracture in comparison with that caused by standard doses of decamethonium made up (if necessary) in normal urine.

IV. Excretion

From biological tests made with urine excreted after intravenous administration of methonium compounds in man and animals, evidence was obtained that the urine possessed marked biological activity. Using hexamethonium as test substance, Zaimis (185) was able actually to isolate the unchanged compound from the urine and failed to discover any degradation product.

There is no reason to suspect, so far, that demethylation or other metabolic attack takes place in the body; so that it can be assumed, on the knowledge so far gained, that the urinary output of methonium compounds represents a measure of the amount absorbed when the drug is given by mouth, and this assumption has been fairly widely made.

After parenteral administration into man or animals, decamethonium and hexamethonium are excreted in the urine fairly rapidly, and 80–90% or more of the injected dose can be recovered (81, 120, 178, 185). The rate of excretion of decamethonium and hexamethonium is such that, in human volunteers, about 60% of the drug given has appeared in the urine 3 hours after intravenous injection, and 90% of it or more in 24 hours. An estimate of the clearance rate of hexamethonium has been made, and found to be close to that of inulin, so that the renal excretion of hexamethonium is mainly due to filtration (183).

In patients with impaired renal function, urinary excretion is slowed and the clinical effects are prolonged for as much as 24 hours after a single intravenous dose. Similarly in the nephrectomised cat under chloralose anaesthesia, the blood level of hexamethonium after an intravenous dose falls very much more slowly than in the normal animal (129). It seems clear that renal excretion is the only important route of elimination of the drug.

Absorption and Distribution

Decamethonium is poorly absorbed by mouth in animals, and roughly 50–100 times as much must be given to produce an effect comparable, at its peak, with that of a given intravenous dose (137). By the subcutaneous and intramuscular routes, about two to three times the intravenous dose is required to produce an equal degree of paralysis, although the duration of paralysis is lengthened when the former routes are used. No studies of this sort have been made in man.

Hexamethonium likewise is poorly absorbed by mouth (81, 120a). When the urinary excretion is used as an index of the amount absorbed, absorption may range from 0.2% to 34% of the dose given, usually about 5–10%. This corresponds with the clinical observation that roughly 10 times more must be given by mouth to obtain the same effects as with parenteral dosage. The part not absorbed is discharged with the faeces, and the total methonium recovered from faeces and urine after an oral dose accounts for at least 60–70% of the drug given (81, 120a). This reinforces the evidence that the drug is little or not at all metabolised in the body.

The factors controlling absorption are still obscure; but there is evidence (81) that some salts of hexamethonium are more readily absorbed from the alimentary tract than others. There is also corresponding clinical evidence that the halides differ in their relative activity by oral administration in depressing spontaneous gastric secretion (98). This is a puzzling observation since the salts when dissolved in the intestinal fluids must be completely ionized, so that the fates of hexamethonium ion and the anion accompanying it should be independent. Absorption is more complete when the drug is given on an empty stomach (81). Again this is confirmed by clinical evidence that fasting patients are more sensitive to the hypotensive action of hexamethonium (98).

After parenteral administration, the concentration of hexamethonium in the blood corresponds to a solution in a volume about 10–30% of the total body volume (121), and is probably to be regarded as passing only into the extracellular space. The view that hexamethonium cannot readily penetrate the cell membrane is concordant with its very slow entry into the cerebro-spinal fluid and into the red blood cell (129), with the manner of its renal excretion by glomerular filtration without secretion or reabsorption, and with its poor absorption from the alimentary tract. Decamethonium, like *d*-tubocurarine, fails to pass the placental barrier. Hexomethonium, however, passes fairly rapidly and, after prolonged administration, the concentration in the amniotic fluid may rise considerably above that in the maternal blood; it seems probable that the foetus secretes hexamethonium in its urine, and that there is a complete cellular barrier separating the maternal circulation from the amniotic fluid into which the urine passes (180–182).

The extracellular existence of hexamethonium follows from its quaternary nature. It is generally accepted that, compared with free bases, ions penetrate cell walls very slowly. Salts of quaternary nitrogen in aqueous solution can only exist in the ionized form, so that such slowness of permeation into cellular elements of the body is to be expected. It is further likely that other ganglion blockers will be of the same character, since they must be similar to acetylcholine (itself a quaternary salt) in order to be able to compete with it satisfactorily. It is probable therefore that most ganglion blockers will be poorly absorbed by mouth, will dissolve in the extracelullar fluid and will be excreted at a rate comparable with that of inulin. The major difficulty about absorption in clinical practice is not so much that it is slow as that it is unpredictable. If ways were found to render absorption more constant, this would do much to overcome the difficulties of oral administration.

V. Neuromuscular Action of Methonium Salts

Decamethonium first received attention as being the most active member of the series in causing neuromuscular block. In its main features this action is superficially like that of drugs such as *d*-tubocurarine, and it satisfies the traditional criteria for curare-like action first laid down by Claude Bernard. In an animal in which sufficient decamethonium has been given to abolish the response of the muscle to excitation of its motor nerve, nervous conduction remains normal, and the muscle can still be excited directly by electrical stimulation. The further criterion, imposed by the theory of chemical transmission, that acetylcholine release should continue during paralysis by decamethonium, is also satisfied. It is this property of neuromuscular block in which the practical usefulness of the drug has been found, and it has proved a competent substitute for *d*-tubocurarine in clinical practice, offering the advantages of high activity, freedom from histamine-releasing and ganglion-paralysing activity, and easy synthesis without need for biological standardization. The statement sometimes made, therefore, that decamethonium is a "curare-like" drug is true in so far as it does indeed produce a true neuromuscular block, and, in clinical practice, useful muscular relaxation.

It has turned out, however, from the earliest experiments that there are numerous and fundamental differences between the actions of decamethonium and those of *d*-tubocurarine and similar drugs (38, 137, 27). The recognition and analysis of these differences has helped to clear up some of the anomalies in the pharmacology of the neuromuscular junction, so that detailed discussion of these differences is necessary, which will lead to a comparison of the modes of action of the two drugs.

1. Modes of neuromuscular block

By neuromuscular block was meant originally, as we have just seen, a motor paralysis which did not involve loss of excitability of nerve or muscle. With the advent of the theory of chemical transmission, offering for the first time a rational basis for the action of curare, the further criterion, that the drug producing neuromuscular block should not prevent the release of acetylcholine, represented a still more delicate test that the drug concerned was not affecting nervous structures. At this time the production of neuromuscular block was usually typified by the action of drugs like curare and much evidence accumulated that they exerted their effects by antagonizing the action of acetylcholine at the motor endplate, as was originally postulated by Dale, Feldberg and Vogt (41).

In recent years the chemical theory of neuromuscular transmission has been elaborated and has become generally accepted. The usual picture of the transmission process is as follows: a nerve impulse traverses the nerve terminations, causing the release of acetylcholine on to the closely adjacent motor endplate. The motor endplate becomes depolarized to a degree sufficient to excite (by electrical spread) the adjacent muscle membrane and so initiates a propagated action potential with accompanying muscular contraction. The release of acetylcholine is very rapid, and its destruction, by cholinesterase concentrated at the endplate region, is also rapid so that the depolarization of the endplate, recognized electrically as the "endplate potential", is a very transient phenomenon, complete in a few milliseconds. After this interval the junction is ready to transmit another impulse. In normal transmission, therefore, the released acetylcholine produces an endplate potential which, as soon as it has reached a certain threshold value, is swallowed up in the much larger propagated muscle action potential. In the curarized muscle the endplate potential is progressively reduced to below this threshold value, until propagation ceases and the muscle contraction is abolished; the further action of curare can only now be recognized experimentally in the continuous gradual depression of the endplate potential.

This view of neuromuscular transmission is also essential to the understanding of a second mode of neuromuscular block, which (in anticipation of our later discussion) we shall refer to as block by depolarization and shall contrast in detail with block by competition. In such block, produced for instance by decamethonium in the cat, the pharmacological lesion of the transmission process is not that of diminution of transmitter action but of exaggeration of such action, for drugs producing block of this kind act by virtue of an ability to produce a persistent depolarization of the endplate, from which follows an electrical inexcitability of the endplate region sufficient to prevent endplate potentials otherwise adequate to their task from successfully exciting the adjacent muscle membrane. Although the ultimate defect in transmission blocked in this way is an electrical one, this implies no qualification of the chemical theory of transmission, for it is the specific chemical sensitivity of the endplate region which enables the prolonged depolarization produced by these agents to occur. In fact the existence of this mode of block emphasises the significance (already pointed out in the early papers) of the presence of cholinesterase close to the point of acetylcholine action, in such a way as to prevent persistent depolarization occurring naturally and inducing block during normal activity.

2. Characteristics of competitive neuromuscular block

There are a number of characteristic features of block by substances raising the endplate threshold to acetylcholine. A large number of substances of this sort, naturally occurring or synthesized, are known, but there is no doubt that *d*-tubocurarine must be chosen as the representative of this group since it is by far the most thoroughly studied. (It must be remembered, however, that there are indications (114, 131) that a small part of its activity is due to properties other than that of competition with acetylcholine, and the ideal representative has probably yet to be found.)

1.) The action of *d*-tubocurarine at the neuromuscular junction is simply that of pure competition; this results in a single uniform picture of neuromuscular paralysis. The course of the paralysis is similar in all species and with all muscles and is never preceded by potentiation of the twitch nor accompanied by contracture. The scattered exceptions to this statement [evidence of contracture of the dog's denervated gastrocnemius (114) and of denervated rats triceps (95)], do not significantly alter this generalization.

2.) The muscle partially paralysed by *d*-tubocurarine cannot maintain a tetanus at its initial strength. Although, in a lightly curarized muscle, the tension may initially be comparable to that of a normal muscle, it falls rapidly thereafter to a much lower level. As the dose of curare increases and as the frequency of tetanisation increases, the ratio of the subsequent contraction height to the initial tension becomes smaller and smaller, and finally becomes zero even though there may still be a fairly vigorous initial twitch. The cause of this flagging tetanic contraction is not established, although no doubt a waning of the acetylcholine output per nerve volley as the tetanus proceeds is an important factor. It constitutes, however, an important aspect of the action of curare-like substances and contributes, for instance, to the greater depression of tetanic activities (such as respiration) than of muscle twitches (141).

3.) Muscles vary appreciably in their sensitivity to *d*-tubocurarine. It has long been known that ocular, cranial and bulbar muscles and those of the hand are outstandingly sensitive. In the cat, likewise, soleus is much more sensitive than tibialis, and the respiratory muscles resemble soleus (141).

4.) Block by curare is antagonized by a wide range of drugs and procedures, including anticholinesterases, adrenaline, asphyxia, potassium, previous tetanisation, acetylcholine and other depolarizing drugs, phenol, and the *m*-OH phenylalkylammonium compounds (for references, see 94, 126, 128).

5.) Block by curare is intensified by substances which raise the endplate threshold to acetylcholine, such as ether anaesthesia, atropine (in large doses) and other substances producing neuromuscular block in the same way as *d*-tubocurarine (137, 141, 143).

6.) The presence of curare diminishes the effect of depolarizing drugs, if administered beforehand, and reverses it if given subsequently (38, 112, 137).

7.) If cathodal current is passed at the endplate region of a partially curarized muscle, the intensity of the block is instantly diminished and normal transmission may be restored. Anodal current, on the other hand, intensifies the paralysis (*cf.* 31a).

3. *Characteristics of neuromuscular block due to endplate depolarization*

We have taken decamethonium in its action on cat, avian and frog muscle as the best representative of drugs producing depolarization block, and in describing its effects on these muscles we shall be illustrating the diverse appearances of the same basic process.

Neuromuscular block by depolarization of the endplate presents a complex picture, for there is always detectable a sequence of neuromuscular excitation followed by depression of transmission. The manifestations of this sequence vary with different muscle preparations and with different species so that the same process may give rise to a situation in which either repetitive firing, or contracture or block of transmission predominates.

a) *Cat muscle.* The paralysing action of decamethonium in the cat is usually heralded by a brief period of fasciculation of the muscle and potentiation of the single twitches. With small doses, such fasciculations may be all that is seen. If the drug is injected close intra-arterially into the tibialis, a twitch of the muscle may be produced by small doses, 3–5 times more than is required for a similar action by acetylcholine. All these reactions are due to repetitive discharge from the neuromuscular junction either spontaneously or in response to single shocks (137). The electrical changes accompanying this are discussed later, together with the changes accompanying complete block.

An analysis of these stimulant effects by Zaimis (186) established that they cannot be attributed (as had been postulated previously) to the weak antiesterase action of decamethonium, and emphasized the analogies between decamethonium and acetylcholine. The evidence against an action through anticholinesterase activity was (1) that the stimulant action was still demonstrable in the presence of a known anticholinesterase in concentration sufficient to inactivate all the enzyme; (2) that the stimulant action did not parallel the antiesterase action when these were compared in a series of compounds, including tetramethylammonium; (3) that the stimulant effects were transient and different from those elicited by anticholinesterases in many respects. This conclusion receives corroboration from the fact that the powerful antiesterase, TEPP, in doses much greater than necessary for complete cholinesterase activation, fails to produce a twitch of the cat's tibialis when injected close intra-arterially (50).

Further evidence that the potentiation of the twitch and the spontaneous fasciculations are not at all due to the weak antiesterase activity but to the active acetylcholine-like action of decamethonium was obtained from denervated cat muscle. In small doses decamethonium produces a twitch if given close intra-arterially, the contraction being associated with propagated action potentials, and in larger doses causes typical contractures during which no more rapid action potentials can be detected. These effects are qualitatively identical with those produced by acetylcholine, but cannot be produced by anticholinesterases (186).

Depolarization of the Motor Endplate (30, 31a). The endplate depolarization by decamethonium, as by any other depolarizing drug or by electrical stimulation, is initially associated with excitation or increased excitability. It is during this phase that spontaneous fasciculation and potentiation of the twitch with repetitive firing to single nerve shocks are seen. But as the depolarization persists, the phase of increased excitability passes over into inexcitability, The succession of cathodal excitation by cathodal block as cathodal current continues to flow is, of course, familiar in peripheral nerve. It may be readily reproduced by the application of a cathode to an endplate region of a normal, or better still, a partially curarized, muscle. The sequence of endplate facilitation and endplate inexcitability then reveals itself by a period of enhanced transmission followed by onset or intensification of neuromuscular block.

During neuromuscular block by decamethonium, as well as during its stimulant action, it is only the endplate region and its immediate environs that is depolarized, and even after the largest doses and with the lapse of hours, the membrane potential of muscle fibre away from the endplate region remains unaltered. The area of depolarization in the endplate region spreads slightly with time to involve the immediately adjacent muscle membrane, and it is during this process that electrical inexcitability develops. This inexcitability, greatest at the point of maximum depolarization, can be revealed in three ways: by a raised threshold to artificial electrical stimulation; by the block, at the endplate region, of the passage of a muscle action potential along the muscle; and by the raised propagation threshold at which an endplate potential will just set up a muscle action potential. In consequence of this depolarization and electrical inexcitability, the endplate potential is set up in the middle of an area of inexcitable membrane, and further, the potential itself is diminished in so far as the membrane is already partly depolarized. From these causes results the neuromuscular blocking action. The block does not bear a constant relation to the depolarization, but it is deeper the longer the depolarization has lasted; this is probably because, as just mentioned, the depolarization spreads slightly with lapse of time by discharging the adjacent membrane so that an everwidening area of inexcitable tissue comes to separate the endplate potential from the nearest normally excitable area of muscle.

None of these electrical changes takes place with *d*-tubocurarine, but they can be reproduced with acetylcholine, or by tetanization in the presence of anticholinesterases. The action of decamethonium thus can be regarded as being in principle exactly the same as that of acetylcholine, save for being spread out over a much longer period of time.

The effects produced by decamethonium in the cat appear to be due solely to this endplate depolarization, and are not accompanied by competition with acetylcholine at the endplate. This is shown not only by the evidence already described, but also by further experiments in which anodal or cathodal currents are passed at the endplate regions for brief periods. In the normal and in the curarized muscle, the application of an anode, which hyperpolarizes the membrane, will cause or deepen neuromuscular block, whereas a cathodal depolarization will lessen it (provided it is not maintained for so long as to produce a cathodal block). But in the muscle paralysed with decamethonium, a cathodal current now immediately deepens the paralysis, and it is with an anode, which removes the depolarization, that the block can also be removed. Were the block due to any curarelike action, such a result could not be obtained. The block by decamethonium therefore is strictly attributable to the depolarization, varying in intensity with the degree and extent of the latter, and passing off as the depolarization passes off or is removed.

Neuromuscular block in the cat due to decamethonium has the following further characteristics (46, 101, 128, 137, 141).

1. Under the influence of decamethonium, the tension of a tetanus is well maintained for the duration of the tetanus.
2. Muscles differ in sensitivity to decamethonium as they do to *d*-tubocurarine, but in the opposite direction. Thus tibialis is particularly sensitive in the cat, whereas soleus and the respiratory muscles are resistant.
3. Block by decamethonium in the cat is not easily antagonized. Anticholinesterases, potassium, asphyxia and previous tetanization are ineffective. Adrenaline has diverse effects according to the route of administration.
4. Substances raising the endplate threshold to acetylcholine (such as penta- or or hexamethonium, *d*-tubocurarine, tetraethylammonium, and ether anaesthesia) diminish the activity of decamethonium.

Studies on rat-muscle have also led to the conclusion that decamethonium in this species exerts an "acetylcholine-like" depolarization of the endplate. Some differences in detail from cat muscle remain to be analysed (95, 96).

(b) *Avian muscle* (33, 187). In adult fowls or in chicks an intravenous injection of decamethonium causes a rigid extension of the limbs and retraction of the head. This is a peripheral effect and the shortening of the muscle is a true contracture. If the dose is lethal the animal dies in contracture; if the dose is below the lethal level the recovery when it occurs consists of an abrupt return to normal.

If decamethonium is injected during the recording of maximal motor nerve twitches of a normal gastrocnemius it produces a double mechanical response consisting of a quick initial contraction followed by a prolonged contracture. Electrical recording shows that the quick response is accompanied by an outburst of action potentials which are cut short by the onset of the slow contracture.

Avian muscle gives an identical response to acetylcholine. *d*-Tubocurarine, on the other hand, causes the usual flaccid paralysis in birds, the block being antagonized by anticholinesterases and by tetanus, and (as in mammals) actually antagonizes the action both of acetylcholine and of decamethonium.

(c) *Frog muscle* (137). Decamethonium produces a contracture of the frog's rectus abdominis, which sums with that produced by acetylcholine, and is antagonized by *d*-tubocurarine, hexa- and pentamethonium, and atropine in large doses. It still produces this contracture when all the cholinesterase in the muscle is inactivated. The contracture is relaxed by anodal current and enhanced by cathodal current (60).

4. *Relation of mode of action to differences between decamethonium and d-tubocurarine*

An attempt has been made elsewhere to relate the differences in the picture of neuromuscular block due to decamethonium from that due to *d*-tubocurarine to their respective actions (128). This argument will not be repeated here, but may be summarized as follows: (1) the presence or absence of stimulant actions and the ability or otherwise to produce contracture correspond precisely to the presence or absence of endplate depolarization; (2) the inverse relationship between the sensitivities of a muscle or a species to the two drugs and their mutual symmetrical antagonism follows from their diametrically opposed modes of action; (3) the lability of the block by *d*-tubocurarine is because the magnitude of the endplate local response to released acetylcholine is reduced to levels at which small changes in this local response produce big changes in transmission, whereas the stability of block by decamethonium arises from the fact that the local response is close to maximal and the block is due to inexcitability of the membrane to the endplate region.

The following summary of the differences between the two drugs has been compiled to indicate those which are most useful in identifying the type of action of a given substance, and to bring out the contrasts between the two pharmacological pictures.

	Actions of depolarizing substance resembling decamethonium	*Actions of competitive blocking substance resembling d-tubocurarine*
A. *Cat Muscle*		
1) Mutual interactions	Potentiates and is potentiated by decamethonium Antagonizes and is antagonized by *d*-tubocurarine	Antagonizes and is antagonized by decamethonium Potentiates and is potentiated by *d*-tubocurarine
2) Inverse relationship on different muscles	Cat tibialis more affected than soleus and respiration	Cat soleus and respiration more affected than tibialis
3) Stimulant actions in normal muscle	Potentiation of twitch with repetitive discharge Spontaneous fasciculations	No stimulant action
4) Denervated muscle	Contracture	Antagonism of the contracture produced by a depolarizing drug
5) Stability of block	Stable	Block increased during a tetanus, diminished after it; antagonism by adrenaline, potassium, anticholinesterases, depolarizing substances, asphyxia, phenol
6) Electrical characteristics	Endplate depolarization with inexcitability of endplate region; block intensified by cathode, relieved by anode	No electrical change at endplate, and normal excitability; block intensified by anode, relieved by cathode of short duration
Avian Muscle	Contracture of limb and neck muscles	Flaccid paralysis and antagonism to contracture
Frog Muscle	Contracture	Antagonism to contracture produced by depolarizing agents

In summary, the two forms of neuromuscular block, when seen in pure form in cat, man, bird and frog, may be contrasted in various ways, expressing the same phenomena in different idioms. Block by *d*-tubocurarine-like substances is mediated by competition with acetylcholine, leading to a depression of transmitter action, of contracture, of cathodal excitation, and of excitatory state at the endplate. Block by decamethonium-like substances, on the other hand, is due to an acetylcholine-like action, to abnormally prolonged transmitter-like action, to endplate contracture, to cathodal block, or to persistent endplate depolarization.

5. *Neuromuscular block of intermediate character*

The work on cat, human, avian and frog muscle accounts for the action of decamethonium as being purely that of the production of a persisting depolarization, which can be contrasted with the purely competitive action of *d*-tubocurarine. But work on certain other species shows that decamethonium can cause in these species a neuromuscular block which differs in many ways from the block accompanying pure depolarization in the cat (187). Thus in the monkey there is no initial potentiation of the twitch, the tetanus is poorly sustained, and the block is antagonised both by anticholinesterases and following the tetanus. Further, if one records simultaneously from the tibialis and soleus, a transition from one type of action to the other is seen; thus at first tibialis is most affected and soleus less (just as with decamethonium in the cat), but as doses are repeated the tibialis becomes less sensitive and the soleus more so, until it may be possible to obtain complete block of soleus, with tibialis still unparalysed, just as can be done in the cat with *d*-tubocurarine. This transition may be so rapid with tibialis that the muscle may fail to be paralysed at all by the second injection of a dose which at first produced almost complete paralysis. In order then to produce the same degree of paralysis it is necessary to give four or more times the original dose.

The impression gained from such experiments, which might be thought to provide a typical example of "tachyphylaxis", is that the phenomena seen in the monkey are like those obtained in the cat when an injection of *d*-tubocurarine is interposed between doses of decamethonium. Similar results have been obtained in dogs, hares, guinea-pigs and rats. Thus the muscles of different species may have widely divergent properties, and the mode of action of a quaternary molecule is determined not only by its own structure but also by the properties of the muscle concerned. The analogy between this "tachyphylaxis" and the effect of interposing *d*-tubocurarine suggests that decamethonium may have a dual mode of action in these species. For instance, it is possible that while the molecules of decamethonium at first adhere in the specific way necessary to produce depolarization of the endplate, their grip subsequently changes so that from a depolarizing substance decamethonium becomes a competitive inhibitor.

A similar transition from the picture of depolarization block to that of competitive block can be seen, with higher members of the methonium series, in the cat and with avian muscle. Thus, after an injection of tridecamethonium into the chick, contracture follows immediately on the injection, but slowly there ensues a stage where the legs are contracted but the head is paralysed, until finally there appears a typical flaccid paralysis. Similarly, in the cat, successive injections of tridecamethonium lead to an increasing refractoriness to paralysis of tibialis and an increasing relative effect on soleus.

6. Variation of potency of decamethonium with species

One of the outstanding problems raised by decamethonium was the varying sensitivity of different species, in contrast with their relatively uniform reaction to *d*-tubocurarine. The observations just described provide a partial explanation. In species where depolarization is the only mode of action of decamethonium (cats, birds), sensitivity is great. It is only when a competitive blocking element appears that the muscle becomes more resistant to decamethonium. It may readily be assumed that under such circumstances decamethonium is interfering with its own depolarizing blocking action. Such a view places the problem of the species difference in a very different perspective, and the question becomes that of why the muscle of different species should change the character of the action of decamethonium, rather than why decamethonium should have a greater affinity for one endplate region than another.

7. Actions of decamethonium in man

Decamethonium has proved a satisfactory relaxant in clinical use, for surgical operations (7, 8, 19, 52, 53, 82, 83, 88, 92, 123, 125, 156, 164, 166, 174, 175, 180), for abating the force of therapeutic convulsions (43, 44, 76, 87, 117), and in the treatment of tetanus (100); the clinical reports cited may be consulted for many details about the actions in man. Responses to decamethonium are not not influenced by age or sex (117). It is generally agreed that decamethonium has a shorter duration of action than *d*-tubocurarine, making it suitable for short-lasting operations, and that when recovery from its effects begins it takes place rapidly without postoperative complications. It gives no signs of histamine-release, and patients in whom *d*-tubocurarine produced bronchospasm have received decamethonium without incident (44, 117). Neostigmine has no antagonistic action to decamethonium in man (76). The suggested use of penta- or hexamethonium as an antidote has not proved successful because the ganglion blocking action of these drugs leads to substantial falls of blood pressure in some subjects but the relatively short action of decamethonium diminishes the need for any antidote. The sparing of respiration which is so prominent in the cat is less noticeable in man, although no quantitative studies have yet been made on anaesthetised subjects. Unna and his colleagues found, in conscious volunteers, that decamethonium affects the respiration more in proportion to its depression of the strength of the hand-grip than does *d*-tubocurarine or its dimethyl ether (171). Davies and Lewis, however, in clinical practice of convulsion therapy, found the respiration less affected by decamethonium, for a given relaxation of the convulsion, than by *d*-tubocurarine (43, 44). The intractable problems of assessing such drugs in clinical practice are discussed by Paton and Zaimis (140), by Unna et al. (172) and by Unna and Pelikan (173). Decamethonium has been used in a wide variety of operations, but some difference of opinion exists as to the operations for which it is most suited; thus some find it inferior to *d*-tubocurarine for intubation (74), whereas others prefer decamethonium to other relaxants (7, 175). It seems probable that these differences correspond to variations in personal techniques and in previous experience with these relaxant drugs. Decamethonium has proved as unsuccessful as other muscle relaxant drugs in the treatment of spasticity (77).

The question arises whether human muscle in its response to decamethonium corresponds more closely to the cat or the monkey. Stimulant effects often precede the paralysis in unanaesthetized subjects (124, 171, 39), and it has been shown by electromyography that these effects are accompanied by a discharge of fibrillary action potentials as well as by motor unit discharges. (39). The ineffectiveness of anticholinesterases against the paralysis produced by decamethonium, the antagonism of a previous dose of *d*-tubocurarine to decamethonium, the capacity of the partially paralysed muscle to sustain a tetanus, and the failure of the tetanic activity of convulsion therapy to accelerate recovery from decamethonium paralysis (156) all correspond to an action by depolarization of the endplate. Pelikan *et al.* have described "tachyphylaxis" to decamethonium in conscious volunteers (143), but this has not been reported clinically (*cf.* 18); indeed Keir has successfully treated a case of tetanus by repeated doses of 2–4 mg. decamethonium every 3–4 hours, giving a total of 135 mg. (100). Finally, the activity of decamethonium in man corresponds closely to that in the cat, at 30–40 μg/kg. It is clear therefore that decamethonium is a depolarizing drug in man, and that whatever may be the evolutionary relationship, human muscle is closer pharmacologically to cat muscle than to that of the monkey.

Two further results of some importance have been obtained from studies by Churchill-Davidson and Richardson of the action of decamethonium on human volunteers (39). First the effect of decamethonium on a muscle varies inversely with the blood flow through it. Thus, in an arm in which sympathetic tone had been released by sympathetic block, it proved impossible to paralyse the abductor digiti minimi by injection of decamethonium into the brachial artery, although sufficient of the drug was given to produce general systemic effects. On the other hand, after an intravenous injection, a chilled limb was more deeply and longer paralysed than a control limb. These results may be important in interpreting some of the details of the action of decamethonium.

Secondly, the same authors have extended the observation by Sellick (157) that the myasthenic patient is not abnormally sensitive to decamethonium, and have further shown that some patients are actually greatly resistant to its action. This represents an important fact about myasthenia, and implies that the threshold of the myasthenic endplate is raised to depolarizing agents. Those theories which attribute the disorder to deficiency in acetylcholine release, or to its abnormally rapid destruction, receive no support from the response of the myasthenic muscle to decamethonium.

8. *Relation to other neuromuscular blocking agents*

We have interpreted the action of the methonium salts as being that of pure depolarization, pure competition, or a mixture of these two; other drugs active at the neuromuscular junction can be classified in a similar way. Among those for which there is good evidence that they are fairly pure depolarizing agents may be included succinylcholine and adipylcholine (23, 33, 133, 176a, and 22, 67–71). A larger number must be regarded as competitive blocking agents, such as the dimethylether of *d*-tubocurarine and the synthetic compounds structurally similar to it (165), the toxiferines (103, 131) and related natural alkaloids, and flaxedil (21). Just as with *d*-tubocurarine, there is evidence that flaxedil may, under special circumstances, have a feeble stimulant action (28, 151a), but its general behaviour and its interactions with depolarizing drugs are typical of competitive block.

Several compounds with a double action comparable to that of tridecamethonium in the cat or decamethonium in the monkey have also been described; for instance, in the alkyltrimethylammonium series studied by Dallamagne and Phillipot (42, 145), it appears that the lower members have chiefly acetylcholine-like properties, depolarizing the endplate (129), whereas the higher members have a double mode of action. Similar results have been obtained with a series of choline esters of adipic acid (67–71), in which methyl groups on the choline nitrogen atoms were successively replaced by ethyl groups. Adipylcholine itself seems to be purely depolarizing, and this is still true when one ethyl substitution is made. With three ethyl substituents on each nitrogen atom it becomes curare-like and antagonistic to the parent compound. But the diethylmethyl derivative is intermediate in its properties, so that it can, for instance, both produce a contracture of avian muscles and also terminate a contracture if it is elicited by the parent substance.

An understanding of the modes of action of these compounds throws considerable light on some of the puzzling interactions between drugs active at the neuromuscular junction. A typical example of such interaction exists in the experiments by Grace Briscoe (25) who found that although neostigmine will relieve block by curare, neostigmine can also produce block itself in the muscle tetanised at a fairly high rate, and that curare, so far from accentuating this block, can actually partially restore normal transmission; she was faced with a situation in which two drugs were capable, under some conditions, of lessening neuromuscular block, and under other conditions of causing or increasing it. But a precisely analogous mutual antagonism between other depolarizing and competitive drugs is now known to exist, and her results are obviously to be interpreted as the consequence of the interaction of the competitive blocking action of *d*-tubocurarine with the depolarization produced in the presence of neostigmine partly by its own direct action, partly by the preservation of acetylcholine released during a tetanus.

VI. Ganglion-Blocking Actions of Methonium Salts

As the length of chain between the quaternary nitrogen atoms is shortened, the neuromuscular action of these salts diminishes very rapidly between the nonane and heptane derivative, to become almost negligible for hexamethonium. Large doses of hexamethonium, can indeed, produce head-drop in rabbits, and, potentiate *d*-tubocurarine. Such vestigial neuromuscular action as it possesses is competitive in nature since it does not depolarize the endplate region and since it antagonizes the action of depolarizing substances such as acetylcholine decamethonium and succinylcholine, as well as the effect of anticholinesterases (49). But a new action now appears, that of paralysing transmission across the ganglionic synapse. This property is at its greatest in the pentane and hexane derivatives, and it is these compounds that have been most closely studied.

1. Site and mode of action

The proof that penta- and hexamethonium specifically paralyse the transmission at the ganglionic synapse has been obtained chiefly with the cat's superior cervical ganglion (142). After administration of a dose sufficient to abolish completely the contraction of the nictitating membrane to preganglionic stimulation, postganglionic stimulation is still fully effective, and the nictitating membrane responds normally to injected adrenaline. Further, hexamethonium in very large doses injected into the arterial inflow of the perfused ganglion fails to reduce acetylcholine output from the preganglionic nerve terminations when the preganglionic trunk is stimulated. These results show that hexamethonium and pentamethonium must act at the synapse itself, rather than on any postganglionic or preganglionic structure. The same conclusion follows from such observations as that hexamethonium will abolish the depression of blood pressure and bradycardia produced by vagal stimulation without affecting that produced by acetylcholine; that it will lower the blood pressure without lessening the pressor response to adrenaline; that it will paralyse salivary secretion to chorda stimulation without reducing that to carbamylcholine, (178), that it will reduce the gastric secretion to stimulation of the vagus nerve, but not to histamine or test-meals (178), and that it will reduce the rapid spontaneous contraction and peristaltic activity of the guinea pig ileum but not the effects of acetylcholine or histamine (57).

The mode of action is that of simple competition. A small dose of hexamethonium antagonises the excitant action of acetylcholine, nicotine, or tetramethylammonium on the blood pressure or superior cervical ganglion; the stimulant action reappears if large doses of those drugs are given but can again be removed by increasing the dose of hexamethonium. There is never any trace of stimulant effects by penta- or hexamethonium even with large amounts injected arterially into the ganglion. No depolarization of the ganglion can be detected if sought for by electrical recording of the potential between the ganglion body and the postganglionic trunk during the action of the drug (130, 132).

Methonium salts are therefore pure competitive blocking agents at the ganglionic synapse. Although highly active in competing with acetylcholine at this site, they lack the other properties which often accompany a relationship to acetylcholine; thus they have trivial neuromuscular blocking action, no atropine-like action, no atropine-sensitive muscarine-like action, and they do not inhibit cholinesterases. Compared with the two other important pure inhibitory ganglionic blocking drugs, *d*-tubocurarine and tetraethylammonium, they are more specific. Thus they lack the histamine-releasing power which complicates the vascular effects of *d*-tubocurarine, and do not produce paraesthesiae, repetitive discharge of nerve, or secretion of adrenaline-like substances from the liver and adrenal glands as does tetraethylammonium. They furnish therefore, drugs of remarkable specificity and are consequently of great use in any analysis in which ganglionic activity may be concerned. Hexamethonium has in fact already proved a useful tool in analysing the action of various drugs on the intestine, in studying the action of ganglionic stimulants and in investigating the question of intestinal adrenergic ganglia (1, 2, 4, 57).

2. Cardiovascular actions

The injection of hexamethonium into the anaesthetized cat causes a fall in blood pressure of fairly gradual onset, depending in size on the initial blood pressure level. With low blood pressures due (for instance) to destruction of the spinal cord, hexamethonium has no depressor effect, even in large doses, from which it may be inferred that it possesses no cardiac depressor action or peripheral vascular action of its own. In the perfused cat's heart, no change in coronary blood flow could be obtained except with very large doses which caused an increase in force of the beat and a rise in flow preceded by a small fall. In the dog's heart, only small and transitory actions on flow were seen, never in the direction of diminution. In the perfused limb, a slight vasodilatation with big doses has been recorded (178). In the rabbit, striking vasodilatation of the ear occurs, which can lead to a 10 to 20-fold increase in heat output from the ear.

Besides the reduction of sympathetic tone produced by penta- or hexamethonium, reflex autonomic activity is modified. In the unanaesthetized dog, in which respiratory arrhythmia of the heartbeat is usually marked, the injection of hexamethonium abolishes the arrhythmia. In cats, the rise in blood pressure due to asphyxia is reduced, presumably in part by paralysis of sympathetic vasomotor action, in part by reduction of the pressor response to splanchnic nerve stimulation (57a). The pressor action of sympathomimetic amines is increased and prolonged after hexamethonium, presumably because the reflex compensatory mechanisms have been inactivated.

3. Gastrointestinal effects

The effects on the motility of the viscera vary with the species (142, 178). In the cat, an injection of hexamethonium leads to increased frequency and strength of contractions, and to spontaneous activity, of the stomach and small intestine. In the rabbit, however, there is a reduction of gastric mobility, and the rabbit's small intestine forms an excellent test object for showing the abolition of the peristaltic reflex by ganglion-blocking methonium salts. These variations probably correspond to the varying preponderance of sympathetic and parasympathetic activity in the two species. Gastric secretion, elicited in the dog by repeated stimulation of the vagus nerve, is also depressed in volume and in acid and pepsin content. The pepsin content is the last to return to normal as the action of the drug passes off.

Salivary secretion, to stimulation of the chorda tympani in the cat, is abolished by relatively small doses of hexamethonium although the response to carbamylcholine is little altered (178).

On the isolated intestine hexamethonium has no effect on the contractions produced by histamine or pilocarpine, and possibly a very slight effect on the contraction to acetylcholine. It reduces or abolishes the actions of nicotine, barium and potassium (57).

4. *Other ganglionic blocking actions*

In the experiments showing that α-β-Ethylal-γ-trimethyl-ammonium-propanediol (2268F) possesses ganglion stimulating action, hexamethonium has been used successfully to inhibit this stimulant effect on the superior cervical ganglion (1). Hexamethonium will abolish the pupillodilator action of cervical sympathetic stimulation. This action is considerably more sensitive to block by hexamethonium than is contraction of the nictitating membrane (121), as it is to botulinum toxin (3).

The hypoglycaemic effect of insulin in dogs (153) and rabbits (105) is potentiated by hexamethonium, so far that a given dose of insulin, previously subconvulsive, sometimes becomes convulsant. The effects of hexamethonium in the unanaesthetized dog (tachycardia, ptosis, relaxation and injection of the nictitating membrane, dryness of the nose and shivering) have been compared to those of sympathectomy.

5. *Other actions of hexamethonium of uncertain mechanism*

The ganglion-blocking action of hexamethonium accounts for almost all the effects it produces in animals and man, and its specificity lends interest to three other actions less easily interpreted:

(1) Antagonism to certain carotid body stimulants. It has been shown that acetylcholine, nicotine and lobeline no longer elicit deepening and acceleration of respiration if hexamethonium is applied to the carotid body. Even with enormous doses of hexamethonium, however, the stimuli of asphyxia or cyanide remain effective (47). There is some discussion as to whether there is an afferent ganglionic synapse on the pathway of the carotid chemo-receptor mechanism, and it is possible that hexamethonium paralyses this. If there is not, then hexamethonium must be regarded as antagonising the action of the cholinergic drugs at a neuronal site other than the ganglion cell.

(2) Antagonism to the stimulant effects of acetylcholine-like drugs on the skin. A parallel situation exists in the skin (48). Although hexamethonium has no local anaesthetic action and gives rise to no disorders of sensation in man, it will prevent the afferent discharge aroused by an injection of acetylcholine or nicotine into an isolated skin preparation. There is no question of a peripheral synapse here, so that hexamethonium must be antagonising acetylcholine-like effects exerted on a peripheral axone.

These two observations give rise to a qualification of the specificity of hexamethonium. Its most obvious action is to prevent the excitation of the ganglion cell by acetylcholine released at the preganglionic nerve endings. But since it prevents excitation of other nervous tissue by artificially applied acetylcholine-like drugs, it may be that its action is not restricted to the ganglion, but is fundamentally to prevent such excitation of nervous structures wherever it can be achieved.

(3) Of interest, therefore, is the third action of hexamethonium, that of central nervous depression. Large doses certainly depress the respiration, particularly in the barbitalized animal, without any neuromuscular block of a degree adequate to account for this (38, 142). It leads to a diminished anaesthetic requirement in man, and causes sleepiness in some subjects (179, 121). Finally, it abolishes nicotine convulsions, but not the tremor induced by nicotine (24, 34, 104). No doubt these actions could be attributed, in whole or in part, to block at a cholinergic synapse in the central nervous system. It will, however, require careful analysis to verify that peripheral block of ganglia cannot indirectly produce such effects, before this speculation can be accepted.

6. *Factors influencing the response to hexamethonium*

Although under standardized conditions hexamethonium has a highly reproducible action, the intensity of its effects may readily vary from one preparation or patient to another according to the conditions.

(a) *Background tone.* If the action studied is the reduction of some spontaneous autonomic function, such as that of the normal vasomotor tone, then hexamethonium will necessarily do no more than remove the background of ganglionic activity concerned. Hence the same dose may give a large fall in blood pressure in a recently anaesthetized cat with high vascular tone, a much smaller one in the same animal after lapse of time, and none at all in the pithed animal. Likewise the effect on the pupil, the heart rate of the alimentary motility depends on the autonomic activity to which the organs concerned are subjected.

(b) *Variations in sensitivity.* Ganglia are not all equally sensitive to blocking agents, and differences occur not only between different ganglia (79) but even, as Langley showed long ago, between different cell groups within a ganglion. With hexamethonium, it is much easier to paralyse the secretion of the salivary gland to chorda stimulation than the contraction of the nictitating membrane to cervical sympathetic excitation (132a) and the latter is also resistant compared to the dilatation of the pupil elicited (121); it needs, indeed, very large doses of hexamethonium to abolish completely the response of the nictitating membrane to brief tetani. Not only do different ganglia respond in different degree to a single drug, but the pattern of responses differs when the drug is changed. Some emphasis was placed on this in the early work, and it appeared possible that the relatively intense effect of pentamethonium on the superior cervical ganglion compared to that on the peristaltic reflex of intestine, in contrast to the intense relative effect of hexamethonium and tetramethonium, reflected a differing relative action on the sympathetic and parasympathetic components of the autonomic system (142). This simple picture has failed to survive, particularly because all agents paralyse salivary secretion first (132a). But it is clear that ganglia do in fact differ, so that the possibility remains open of devising blocking agents directed in their effects to one particular set of ganglia.

(c) *The possibility of maximal block.* It is not always possible to obtain complete abolition of ganglionic transmission, even with large doses. In particular the blood pressure level after hexamethonium never falls to that of a pithed cat; and the difficulty of complete paralysis of the nictitating membrane to a brief tetanus has already been mentioned.

(d) *Intensity and duration of activity.* The effect of hexamethonium on a ganglion depends on the activity of the ganglion, both at the time of administration and previously. Thus a freshly established tetanus may be well-maintained for a brief period, and then wanes considerably. Again it may be shown that as excitation is prolonged, the sensitivity of the ganglion increases. Similarly, the higher the rate of stimulation, the smaller the dose of hexamethonium required to produce a given reduction in transmission.

This relationship between activity and transmission block is very similar to that which exists in the curarized muscle, and in both instances it may be that a dwindling of the acetylcholine output with continued or accelerated frequency of nerve stimulation enables hexamethonium or curare to exert its blocking action more readily.

(e) *Compensatory mechanisms.* The tolerance to the hypotensive actions of hexamethonium which develops in hypertensive patients on regular dosage suggests that the effects of the drug may be distorted by compensatory mechanisms of one sort or another. Reactions of this kind, of course, take place whenever the blood pressure is changed, and hexamethonium, in fact, helps to reveal their occurrence, *e.g.*, the abbreviation and curtailment of the effects of adrenaline by buffer nerve reflexes.

Some care is needed, therefore, in interpreting the precise significance of a given depression of ganglionic activity, and comparisons can only be satisfactorily made at present when the ganglion used, the rate of preganglionic excitation, the duration of activity and the compensatory mechanisms are standardized.

7. *Hypertension*

The increasing recognition that nervous factors play an important part in hypertension has led first to the use of surgical sympathectomy in its treatment and later to the use of drugs which weaken vasoconstrictor tone. Among such drugs, those which paralyse autonomic ganglia offer certain advantages, particularly that the blood vessels are not themselves affected. In the event of overdosage producing too great a hypotension, sympathomimetics can therefore be used as antidotes; this is of course difficult if the hypotension is produced by substances antagonizing the effects of adrenergic nerves or of sympathomimetic amines. Tetraethylammonium was the first ganglion blocking drug to be used, and although its transient action and side effects limited its success, experience with it made it obvious that treatment on these lines was worth investigating. Hexamethonium has proved much more suitable, by reason of its more prolonged and purer action, and extensive clinical trials have been conducted (5, 6, 11, 17, 32, 35–37, 58, 61, 64, 65, 75, 102, 107, 109, 116, 122, 146, 150a 152a, 159, 160–162, 168–170, 177).

The fall in blood pressure produced in hypertensives by hexamethonium is largely due to the removal of abnormal sympathetic constrictor tone and it can be roughly matched by the fall obtained if the central nervous autonomic drive is reduced by sedatives. The effect obtained varies considerably, corresponding no doubt to the relative proportions of the neurogenic and humoral components of the raised blood pressure (*cf.* 6 and 62, 63). In almost every case, however, a reduction in the blood pressure can be obtained if the patient is in the erect posture, because of the depression by hexamethonium of postural vaso motor reflexes. This postural hypotension is an essential element in the use of hexamethonium and is not to be regarded as a side effect.

From the clinical reports already published, particularly those by Campbell and Robertson, Smirk and his colleagues, Turner, Rosenheim, and Finnerty and Freis, there is no doubt that hexamethonium properly used in the right patients can produce considerable symptomatic and objective improvement in cases of hypertension. Patients with the more severe and complicated forms of the disease are those that benefit most. Headache, breathlessness and dizziness are almost invariably improved. Papilloedema and retinal damage usually regress. Cerebral oedema and vomiting can be relieved. Pulmonary oedema may be lessened or resolved (61) and cardiac hypertrophy diminished. In the few cases of toxaemia of pregnancy and eclampsia whose treatment has been described, hexamethonium has proved successful in permitting a live birth or in terminating *status eclampticus* (144, 168). The action of hexamethonium is enhanced by a salt-poor diet.

In milder cases of hypertension with a good prognosis, a full clinical assessment of the advantages to be gained has yet to be made. There are certain difficulties in successful treatment against which therapeutic gain has to be balanced. In the first place oral administration has not proved satisfactory as was at first hoped because of the poorness and irregularity of absorption. Consequently parenteral routes must be used in most (but not all) patients (73, 150a) and an "insulin regime" of subcutaneous or intramuscular injections, developed by Smirk and his colleagues, has proved to be the most generally effective means of treatment so far. Secondly, the dose required by a particular patient is difficult to predict, since the effect depends on a large number of variables discussed below. This means that treatment of a patient requires careful individual adjustment, best initiated in the hospital, and that it needs continuous supervision thereafter. Thirdly, since the action of hexamethonium is due to competitive block of the ganglionic synapse, partial block of ganglia other than those controlling the blood pressure may lead to actions commonly but loosely termed "side effects." The most important of these are due to paralysis of parasympathetic ganglia, *i.e.*, constipation, rarely leading (especially after big oral doses) to ileus (20, 108, 111, 115, 167), difficulty in micturition, and interference with pupillary and ciliary reactions to light and accommodation. Constipation usually responds to liquid paraffin or purgatives and ileus (if allowed to occur) has been successfully treated by neostigmine (20). Pilocarpine (150a) and carbachol (65) have also been used to overcome the effects of parasympathetic blockade. Sometimes the postural hypotension from depression of sympathetic vasomotor reflexes may be so severe as to cause faintness or collapse (10), particularly when the blood pressure is greatly reduced too abruptly. The hypotension responds to sympathomimetic amines, as expected from the mode of action of hexamethonium (14), but infusions of adrenaline have proved dangerous. The use of posterior pituitary extract has been suggested (29). Grob and Harvey (75), after showing that neostigmine failed to diminish this hypotensive effect, drew the conclusion that "the blocking action of hexamethonium does not appear to be the result of competitive inhibition of acetylcholine." All the weight of the evidence, already discussed, is against this suggestion, and their results may well follow from the fact that antiesterases reveal their rather weak action at the ganglionic synapse only under special experimental conditions. Bromism occurred in some patients when hexamethonium bromide was administered, particularly when this treatment was combined with a low sodium diet (89, 107, 150, 163), but this can be avoided by using other salts.

In addition to the therapeutic results of the use of hexamethonium, certain facts have emerged of some general physiological and pathological interest. The first of these is that during the first few weeks of repeated administration the hypotensive effects of hexamethonium usually wane and the dose required to produce the original depressor effect increases, although after this period a regular maintenance dose can be achieved. This "tolerance" is lost again when hexamethonium is withdrawn. Tolerance does not appear to be due to any increased rate of excretion of hexamethonium or to the development of a "detoxicating" mechanism (121). Its significance is obscure, but it may be compared either to the recovery of vascular tone after sympathectomy or to a compensatory development of humoral vasoconstrictor activity. It must be remembered, however, that tolerance develops to the effects of most drugs when these are given over a prolonged period.

A further important finding, already briefly mentioned, is the variability of the response of human beings to the drug. At least four factors may contribute to this: 1) differences in the postural reaction, depending probably on the vigour of the reflexes, on the peripheral blood flow and on the muscle tone of the individual; 2) differences in the intensity of sympathetic tone; 3) differences in absorption of hexamethonium from the intestine or even (to some extent) from its subcutaneous or intramuscular injection site; 4) differences in its distribution throughout the body and in the renal blood-flow on which its excretion depends. Quite widely divergent curves of concentration of hexamethonium in the plasma against time may be obtained, even with normal subjects (121).

The variability of response in man thus reflects not so much an irregularity in the action of hexamethonium on ganglia but the differences in sympathetic tone, in reflex function, and in absorption, distribution and excretion of the drug. It must be expected to occur with any drug with the same mode of action.

8. Reduction of bleeding during surgical operations

An important innovation is the use of ganglion-blocking agents for reducing bleeding at surgical operation (45, 54, 55, 56, 91, 106, 158). It had been earlier shown that lowering the blood pressure by spinal anaesthesia, or by temporary withdrawal of blood diminished considerably the bleeding during plastic operations and ear, nose and throat surgery. Hexamethonium has proved to be equally or more effective for this purpose, and considerably easier to handle. The desired reduction (to c. 60–70 mm. mercury) cannot be produced by hexamethonium alone in a supine patient, and postural assistance is usually employed. After sympathetic tone is reduced or abolished by hexamethonium, blood can be pooled in the vessels of a dependent part of the body (usually the legs or viscera), thus lessening the cardiac output (177) and hence bringing the blood pressure down to the desired level. Suction applied to the legs has also been used for this purpose (152). In addition, the operative field can be further exsanguinated by raising that part of the body in which it lies above the rest, so that the blood is drained away from it. When the operation is over and the effect of the drug passes off, there appears to be no increased liability to reactionary haemorrhage. Presumably clotting takes place in the relaxed vessels just as in vessels with normal tone, and with recovery of tone and contraction of the vessel the clot might even become more firmly held.

This technique has been particularly successful in plastic surgery, ear, nose and throat surgery, in thyroidectomy and in mastectomy, and it may greatly facilitate and abbreviate operation. Satisfactory results have also been reported in thoracic, abdominal and pelvic operations.

The doubt naturally occurs whether the blood supply to the brain, heart and kidneys remains adequate during such hypotension. It has been shown that the renal circulation at least is not greatly reduced, although anaesthesia itself may lower it considerably (119). This result implies that compensatory vasodilatation in the kidney occurs. Considerable attention has been paid by anaesthetists to the state of the heart, particularly in older patients subjected to this technique, but no electrocardiographic signs of coronary ischemia could be found in either young or old patients with pressure reduced to 60–70 mm. mercury (55). Hexamethonium has proved of great assistance to the neurosurgeon, diminishing haemorrhage and improving the visibility (particularly with vascular tumours), lessening intradural pressure and cerebral oedema and greatly accelerating the operation. Given careful control of posture, no cerebral damage occurs and normal encephalograms have been obtained throughout such operations (78, 176). Attention has been drawn to the danger of anoxic liver damage, with release of V. D. M., if the blood pressure falls below 60 mm. (26).

Apart from the premeditated reduction of haemorrhage during the whole of an operation, hexamethonium has been employed to control unexpected haemorrhage; thus bleeding from a pulmonary vein and from a gastric artery were successfully and rapidly checked (80, 148).

The hypotensive technique has proved remarkably promising and has posed many interesting physiological problems (127). The fact that so low a pressure can be sustained for a period of hours with little deleterious effect is remarkable, and provides a complete contrast to the effects of similar hypotension due to haemorrhage. The difference is no doubt due to the fact that, whereas with hexamethonium capillary blood flow may well be normal or even increased, after haemorrhage it will certainly be reduced. But until more work has been done in this field, it is difficult to be certain of all the factors involved in the reduction of blood pressure and of haemorrhage, and of their relative importance. For instance the hypotheses that relaxation of venous tone contributes to the postural effects, that the capillary pressure does not fall as the blood pressure falls and that the raised operative field is more or less ischaemic, have not been directly established.

Although hexamethonium given before operation diminishes or prevents haemorrhage, it may, when given after a haemorrhage, accentuate considerably the fall in blood pressure resulting from the loss of blood (55, 66). If this occurs, however, the blood pressure can be promptly restored by blood transfusion. Similarly in the cat, small doses of hexamethonium greatly increase the depressor effect of haemorrhage (129). Such experiments bring into great prominence the role of nervous factors in maintaining a stable blood pressure in the face of alterations of blood volume. For practical purposes, it is essential to grasp that, after hexamethonium, the patient's circulatory needs have to be cared for by the anaesthetist just as after a muscle relaxant artificial respiration must be available, and that in both situations the normal neural control is suspended. Given an understanding of this point, however, there seems to be no reason why "controlled circulation" should not be as safe as "controlled respiration."

9. Actions on the human alimentary tract

Investigations into the action of hexamethonium on gastric function were initiated by Kay and Smith (97) who showed that hexamethonium given intramuscularly to fasting patients could depress the volume and acidity of the gastric secretion occurring spontaneously or in response to insulin, to the point of achlorhydria. Gastric secretion elicited by histamine, alcohol or meat extract was not affected. Depression both of the volume and pepsin content of the nocturnal "interdigestive" secretion occurs, and the reduction is of the same magnitude or greater with hexamethonium as with *l*-hyoscyamine or with vagotomy (151). Later experiments showed that similar effects could be obtained by oral administration of hexamethonium, although less consistently than with parenteral administration. Gastric motility is also inhibited, sometimes completely, and for many hours (98, 99). Slowing of the emptying of the stomach and small intestine has been demonstrated by X-ray after barium meals. The effects both on secretion and on motility are less pronounced when the stomach is full (51). Clinically, hexamethonium often relieves the pain of peptic ulcer, and a careful preliminary trial suggests that its actions in depressing secretion and motility may actually accelerate healing (155). Full assessment of its usefulness must, however, wait for a clinical trial prolonged enough to allow control of the well-known spontaneous remissions in the disease.

10. Comparison of tetra-, penta- and hexamethonium

In the original pharmacological studies, attention was drawn to the apparent difference in relative activity of the various ganglion-blocking salts on sympathetic and on parasympathetic ganglia. It was hoped that such differences might be exploited clinically where the ganglia concerned differ (*e.g.*, hypertension and peptic ulcer), so that for instance hypotension could be avoided in the treatment of peptic ulcers, or intestinal effects avoided in the treatment of hypertension. Tetra-, hexa- and pentamethonium furnished a series with increasing effect on the sympathetic superior cervical ganglion of the cat relative to the action on the parasympathetic peristaltic reflex, such that if hexamethonium were regarded as equally active in both tests, then pentamethonium is 2½ times more active on the superior cervical ganglion than on the peristaltic reflex, and tetramethonium is 3 times less active. In practice, however, these differences have not proved large enough to be clinically useful, and no evidence has been obtained in man that tetramethonium and pentamethonium differ qualitatively from hexamethonium (17, 54, 93, 99). The only difference between these three drugs in man is that pentamethonium is somewhat less active, and tetramethonium much less active than hexamethonium.

11. Other actions of hexamethonium in man

The most valuable effects of hexamethonium therapeutically are the reduction of blood pressure, the diminution of haemorrhage at operation, and the depression of gastric secretion and motility. But many other less important actions have been described. Depression of sympathetic vasomotor tone leads not only to postural hypotension, but also to an increase in limb blood flow (especially of the leg and foot) accompanied by a rise in skin temperature, to a depression of the reflex pressor responses accompanying application of cold to the hand or following the Valsalva manoeuvre, and to a sensitization to the effects of venous congestion of the limbs, or of changes of hydrostatic pressure in an immersed subject (66, 147). These reflex responses, particularly the postural vascular reflexes, seem to be particularly sensitive to hexamethonium, and in normal subjects at least postural hypotension persists long after the blood pressure measured in the supine position has returned to normal.

Hexamethonium causes a dilatation of the pupil, lessens the reaction of the pupil to light and to accommodation, and weakens the accommodation of the lens. In addition there is usually a slight injection of the conjunctiva, and dryness of the eyes. The mouth, throat and larynx are also sometimes noticed to be drier than usual after hexamethonium, and sweating may be considerably decreased (32, 121).

Pentamethonium and hexamethonium have been used in a few cases of peripheral vascular disease (5, 32, 59, 154), but equally or more effective means which do not involve a general ganglionic action are usually available. Hexamethonium has been successfully used in causalgia (149), exerting its action probably by relieving sympathetic vasospasm of the vessels supplying the injured nerve or adjacent regions. A curious observation is that hexamethonium may relieve hiccup (84, 113). It has also been shown to antagonise the stimulating action of lobeline on the carotid body (85).

12. *Toxicity*

In the original studies, when large daily doses of hexamethonium were given intravenously for a month to rabbits, no toxic effects were found. This absence of toxicity has been confirmed in human experience. Despite the large quantities of the drug which must by now have been administered to many patients, for periods of sometimes more than 2 years, no case has yet been described recording damage of the haematopoietic system, liver, kidney or other organs, or sensitization phenomena of any sort. This freedom from toxicity is no doubt attributable in part to its extracellular existence and in part to its physical and chemical inactivity and stability, factors all closely related to its quaternary character.

VII. Other Members of the Methonium Series, and Other Actions

With chain lengths shorter than C4, the series becomes very inactive, and C2 and C3 are surprisingly inert pharmacologically, and are in all respects weaker than tetramethylammonium to which they bear so close a relation. At the other extreme, with chain lengths greater than 10 carbon atoms, activity again falls away, although less rapidly. An anticholinesterase action becomes fairly prominent, *in vitro* at least, although it is always small compared with eserine; its main interest is that for certain members (C12 in particular) the activity is greater against red blood cell esterase than against plasma esterase; most compounds inhibit the latter more readily. A very feeble muscarine-like action also appears, reaching a maximum at C12–C13. The longest chained member tested, C18, is surface-active, and to this is probably attributable a weak antibacterial action against *E. coli*, streptococci and staphylococci in concentrations of 0.3–8 parts per 100,000.

VIII. Specificity, Potency, Site of Action and Chemical Structure

From the theoretical point of view, it is interesting that such simple molecules as hexamethonium and decamethonium should be so specific in their actions. Most of the drugs used in pharmacology for their potency and specificity, being often of natural origin, have had a relatively complex structure (*e.g.*, physostigmine, *d*-tubocurarine, toxiferine I, atropine). This has led chemists to use such complex molecules as their models in preparing drugs of like action synthetically. Of recent years, however, a progressive simplification has taken place which can be seen, for instance, in the development of flaxedil from 8′,8″-diquinolyloxy-1,5-pentane di-iodethylate by Bovet, of neostigmine from eserine by Aeschlimann and Reinert and of sulphonamides from the prontosil dyes. The simplicity of structure of the methonium salts emphasizes this development, although obviously the limit of simplification has been reached in that particular direction. It is notable that with drugs acting at the neuromuscular junction their structure has become steadily nearer that of acetylcholine, just as the successors to the prontosil dyes became more and more like *p*-aminobenzoic acid. All this suggests that, in developing new substances with potent pharmacological actions, it is safer to use as models substances occurring naturally in the body rather than those more complicated alkaloids through which so much physiological knowledge first came to light. It seems further that simplification of the molecule, so far from allowing side actions to multiply, in fact diminishes them; for both hexamethonium and decamethonium compare very favourably with natural alkaloids in their freedom from such actions. This process, of course, can only be taken up to a certain point; the simplest salts, tetraethylammonium and tetramethylammonium, representing completely unencumbered active quaternary radicals, lose the specificity of hexamethonium or decamethonium both as to site and type of action.

Despite the simplicity of these compounds it is still very difficult to relate their activity and specificity to their chemical structure. The literature is full of shipwrecks in the quicksands of 'structure-action-relationship,' and it is obvious that our biophysical knowledge is inadequate to the pharmacological strains placed on it. It is worth while, however, to mention briefly some of the points which a satisfactory theory would have to take into account even when we restrict the problem to the neuromuscular and ganglionic synapse.

Acetylcholine, at the endplate or in the ganglion, reacts not only with the specific receptor surface but also with cholinesterase. A drug resembling acetylcholine can thus reveal its likeness in three ways: by mimicking acetylcholine; by competitively antagonizing it; and by antagonizing the activity of the cholinesterase. We may have, therefore, among the effects of such a drug, excitation or competition at the endplate or in the ganglion, as well as antiesterase activity, quite apart from any peripheral autonomic 'muscarinic' actions of central actions. The theory, therefore, must account for the way in which a drug such as decamethonium or hexamethonium can select, from the protean manifestations of acetylcholine action, that of endplate stimulation or of ganglion competition alone.

The structural factors determining the site of action, the mode of action, and the potency of hexamethonium and decamethonium can be brought out by comparing them with each other and with compounds closely related to them chemically; even with such elementary structures, four factors can be distinguished:—

(a) *Interquaternary distance.* Comparison of hexamethonium with decamethonium shows that the interquaternary distance determines both their site and mode of action, and it is extraordinary that a change which looks so simple in terms of chemical structure should transform so fundamentally the pharmacological action. Even the slighter change from hexamethonium to tetramethonium or from decamethonium to octamethonium produces great reductions in potency. The peaks of activity at C5–C6 and C10 have prompted a good deal of speculation and synthetic chemistry. Among the other series prepared possessing neuromuscular or ganglionic activity, some have a peak of activity corresponding to one of those in the methonium series, but this is by no means the rule. Similar maxima exist among chemotherapeutic agents and in the histamine-liberating aliphatic diamines. Unfortunately there is no certain knowledge as to the actual interquaternary distance in compounds with flexible chains of this sort. There is, therefore, a good deal of doubt as to the interpretation of the significance of the chain-lengths of hexamethonium and decamethonium, and the idea that the length of the chain necessarily corresponds to an interreceptor distance has been questioned (110). Another suggestion is that it corresponds rather to a certain molecular bulk just capable of occluding a certain critical receptor area. Nevertheless, the lengths concerned are so sensitive to alteration that considerable importance attaches to them, and the naive view that they represent some measure of interreceptor distance need not be abandoned.

(b) *Interquaternary structure.* The structural differences between decamethonium and succinylcholine do not lead to very noticeable differences of action apart from the susceptibility of the latter to hydrolysis. But the inclusion of aromatic nuclei between the basic groups has a more radical effect, usually in rendering the drug more competitive in nature and often less specific.

(c) *The central atom of the terminal groups.* It has been shown since the work of Ing and Wright that substitution of nitrogen by sulphur, phosphorus or arsenic leads to a similar but progressively weaker activity. Similarly with the bisonium salts, the bis-sulphonium compounds are distinctly feebler than the corresponding bis-ammonium compounds (176a). There is no rival so far to quaternary nitrogen for biological potency among onium salts.

(d) *The groups substituted on the basic nitrogen.* If the methyl groups in decamethonium are all replaced by ethyl groups the compound is much less active, and becomes a competitive blocking agent as well as acquiring appreciable action in paralyzing ganglionic transmission. A similar transition is known for adipylcholine, which loses its power of producing acetylcholine-like effects at the neuromuscular junction as soon as more than one methyl group is replaced by ethyl (71). The situation seems to be comparable with the result of changes in the molecule of acetylcholine itself; Holton and Ing (90) showed that, although a single substitution of an N-methyl group by an ethyl radical had only a small effect on the activity of the molecule, any further substitution changed it radically.

Even when all these structural considerations are taken into account, however, it is still impossible to state the expected action of a compound. This is particularly obvious with decamethonium, whose expected action cannot be predicted unless the species and even the muscle on which it is tested is known. In other words, the receptor structure too must always be considered, and cannot be assumed to represent a constant framework from animal to animal into which chemical structures of varying ingenuity may be fitted.

IX. Conclusions

The detailed pharmacological analysis of hexamethonium and decamethonium has elucidated fairly completely their site and mode of action. Once this was done, and their remarkable specificity appreciated, they became at once useful research tools in the hands of pharmacologists and physiologists. First one may quote the clear distinction that can be made between the two types of neuromuscular block, each now easily recognized by its own characteristic syndrome of responses. Blocks distinguishable in the same way by deficiency or excess of the excitatory process can be recognized also at the ganglionic synapse. No doubt, in due course, other substances will appear as specific for the central nervous synapse as the methonium salts have been for the neuromuscular junction and ganglionic synapse, and our new knowledge about the peripheral synapses should help greatly in disentangling what is at present a very difficult picture.

Another valuable development has been the addition to our knowledge of the pharmacology of the human organism, which has lagged far behind that of animals. The similarity of human muscle to the muscle of the cat, the different reactions of 'red' and 'white' muscle, the resistance of the myasthenic to decamethonium, the effects of hexamethonium in man and particularly the fact that the blood pressure may be safely reduced by it to levels commonly regarded as exceedingly dangerous,—all these represent stimulating observations from which fruitful developments may be expected. It is beyond the scope of this review to comment in general on the clinical usefulness of quaternary salts in the future. But whatever new discoveries are made, whether in extending the use of the methonium salts themselves or in developing new compounds with a more advantageous balance of actions, the specificity, potency, versatility and freedom from toxicity of the methonium compounds must provide a great encouragement and guide to research in this direction.

At the outset of this review some mention was made of the background to the study of compounds such as the methonium series. The growth of electrical recording methods, of synthetic chemistry, and of clinical trial and investigation have all played their part. But reflection on the fundamental ideas involved and the experimental techniques required show that among the developments leading to this therapeutic harvest, the bringing to fruition of the theory of chemical transmission at the neuromuscular and ganglionic synapses must occupy pride of place.

REFERENCES

1. AMBACHE, N. The nicotine action of substances supposed to be purely smooth-muscle stimulating. (a) effects of a-β-ethylal-γ-trimethyl-ammonium-propanediol (2268 F) upon skeletal muscle and ganglion cells. J. Physiol., **110**: 145-163, 1949.
2. AMBACHE, N. Unmasking, after cholinergic paralysis by botulinum toxin, of a reversed action of nicotine on the mammalian intestine, revealing the probable presence of local inhibitory ganglion cells in the enteric plexuses. Brit. J. Pharmacol., **6**: 51-67, 1951.
3. AMBACHE, N. Botulinum toxin and the superior cervical ganglion. J. Physiol., **116**: 9 p. 1952.
4. AMBACHE, N. AND EDWARDS, J. Reversal of Nicotine action on the intestine by atropine. Brit. J. Pharmacol., **6**: 311-317, 1951.
5. ARNOLD, P., GOETZ, R. H. AND ROSENHEIM, M. L. Effect of pentamethonium iodide on the peripheral circulation. Lancet, **2**: 408-410, 1949.
6. ARNOLD, P. AND ROSENHEIM, M. L. Effect of pentamethonium iodide on normal and hypertensive persons. Lancet, **2**: 321-323, 1949.
7. BAILEY, P. J. AND MURPHY, F. J. Syncurine in endotracheal intubation. Anesthesiology, **12**: 63-66, 1951.
8. BALABAN, I. E. AND BETON, J. A. The properties and reactions of some methonium compounds. J. Pharm. & Pharmacol., **3**: 360-366, 1951.
9. BALABAN, I. E., LEVY, M. B. AND WILDE, B. E. The properties and reactions of decamethonium iodide and hexamethonium bromide. J. Pharm. & Pharmacol., **1**: 603-606, 1949.
10. BALL, J. D. Dangers of hexamethonium. Lancet, **2**: 650 1950.
11. BANTON, A. H. Methonium compounds in hypertension. Lancet, **1**: 527, 1951.
12. BARBER, H. J. AND GAIMSTER, K. Hexamethonium bitartrate and other hexa-alkonium salts. J. Pharm. & Pharmacol., **3**: 663-669, 1951.
13. BARBER, H. J. AND GAIMSTER, K. A sparingly soluble hexamethonium salt and its applications, some observa-

tions on allied quaternary ammonium salts. Proceedings of the Society of Chemical Industry (Fire Chemicals Group) February, 1952.

14. Barcroft, H., Churchill-Davidson, H. C. and Swan, H. J. C. Noradrenaline in hypotensive states. Lancet, 1: 854, 1951.

15. Barlow, R. B. and Ing, H. R. Curare-like action of polymethylene bisquaternary ammonium salts. Nature, 161: 718, 1948.

16. Barlow, R. B. and Ing, H. R. Curare-like action of polymethylene bis-quaternary ammonium salts. Brit. J. Pharmacol., 3: 298-304, 1948.

17. Barnett, A. J. Comparison of pentamethonium and hexamethonium bromide. Lancet, 1: 1415, 1951.

18. Barry, C. T., Stratton, J. and Sutherland, A. R. Cumulative action of decamethonium iodide. Lancet, 1: 507-508, 1951.

19. Bernstine, M. L. Some basic principles of geriatric anesthesia. Geriatrics, 6: 40-44, 1951.

20. Bourne, G. and Hosford, J. Methonium compounds in hypertension. Lancet, 1: 527, 1951.

21. Bovet, D., Depierre, F., Courvoisier, S. and de Lestrange, Y. Recherches sur les poisons curarisant de synthese, IIme partie. Ethers phenoliques à fonction ammonium quaternaire. Action due tri-iodoethylate de tri(diethylaminoethoxy) benzene. (2559 F.). Arch. Internat. de pharmacodyn., et de thérap., 80: 172-188, 1949.

22. Bovet, D., and others. Curari di sintesi. Rendiconti Ist. Sup. di Sanita., 12: 1-264, 1949.

23. Bovet, D., Bovet-Nitti, F., Guarino, S., Longo, V. G. and Fusco, R. Recherches sur les poisons curarisants de synthese. IIIe Partie: Succinylcholine et derives aliphatiques. Arch. internat. de Pharmacodyn. et de thérap., 88: 1-49, 1951.

24. Bovet, D. and Longo, V. G. The action on nicotine-induced tremors of substances effective in Parkinsonism. J. Pharmacol. & Exper. Therap., 102: 22-30, 1951.

25. Briscoe, G. Changes in muscle contraction curves produced by drugs of the eserine and curarine groups. J. Physiol., 93: 194-205, 1938.

26. Bromage, P. R. Effect of induced vascular hypotension on the liver. Lancet, ii, p. 10-12, 1952.

27. Brown, G. L., Paton, W. D. M. and Vianna Dias, M. The depression of the demarcation potential of cat's tibialis by bistrimethylammonium decane diiodide (C10). J. Physiol., 109: 15P, 1949.

28. Bülbring, E. and Depierre, F. The action of synthetic curarizing compounds on skeletal muscle and sympathetic ganglia both normal and denervated. Brit. J. Pharmacol., 4: 22-28, 1949.

29. Burn, J. H. Methonium compounds in hypertension. Lancet, 1: 642, 1951.

30. Burns, B. D., Paton, W. D. M. and Vianna Dias, M. Action of decamethonium iodide (C10) on the demarcation potential of cat's muscle. Arch. Sci. Physiol., 3: 609-612, 1949.

31. Burns, B. D. and Paton, W. D. M. Depolarization of the motor end-plate by decamethonium and acetylcholine. J. Physiol., 115: 41, 1951.

32. Burt, C. C. and Graham, A. J. P. Pentamethonium and hexamethonium iodide in vascular disease and hypertension. Brit. M. J., 1: 455-460, 1950.

33. Buttle, G. A. H. and Zaimis, E. J. The action of decamethonium iodide in birds. J. Pharm. & Pharmacol., 1: 991-992, 1949.

34. Cahen, R. L. and Lynes, T. E. Nicotinolytic drugs. I. Drugs inhibiting nicotine-induced tremors. J. Pharmacol. & Exper. Therap., 103: 44-53, 1951.

35. Campbell, A. J. M., Graham, J. G. and Maxwell, R. D. H. Treatment of hypertension by oral methonium compounds. Brit. M. J., 1: 251-254, 1952.

36. Campbell, A. and Robertson, E. Treatment of severe hypertension with hexamethonium bromide. Brit. M. J., 2: 804-807, 1950.

37. Campbell, A. and Robertson, E. Methonium compounds in hypertension. Lancet, 1: 797, 1951.

38. Castillo, J. C., Phillips, A. P. and De Beer, E. J. The curariform action of decamethylene-1, 10-bis-trimethylammonium bromide. J. Pharmacol. & Exper. Therap., 97: 150-156, 1949.

39. Churchill-Davidson, H. C., and Richardson, A. T. Decamethonium iodide (C10): some observations on its action using electromyography. Proc. Roy. Soc. Med., 45: 179-186, 1952.

40. Churchill-Davidson, H. C., and Richardson, A. T. The action of decamethonium iodide (C10) in myasthenia gravis. J. Neurol. Neurosurg. & Psych., 15: 129-133, 1952.

41. Dale, H. H., Feldberg, W., and Vogt, M. Release of acetylcholine at voluntary motor nerve endings. J. Physiol., 86: 353-380, 1936.

42. Dallemagne, M. J., and Philippot, E. Synergies et antagonisms a la jonction neuro-musculaire. III. Action de sels d'alkyl-tri-methyl-ammonium chez l'oiseau. Arch. internat. de physiol., 59: 374-376, 1951.

43. Davies, D. L. Substitutes for curare. Lancet, 1: 1091, 1950.

44. Davies, D. L., and Lewis, A. Effects of decamethonium iodide (C10) on respiration and on induced convulsions in man. Lancet, 256: 775-777, 1949.

45. Davison, M. H. A. Pentamethonium iodide in anaesthesia. Lancet, 1: 252-253, 1950.

46. Depierre, F. Pharmacodynamie.—Antagonisme entrè l'action curarisante (triiodo ethylate de tri (-diethylaminomethoxyl) -1,2,3 benzene) et l'action curariforme (iodure de tetramethyl-ammonium acethylcholine, dibromure de decamethylene bis-trimethylammonium et prostigmine). Compt. rend. Acad. sc., 232: 768-770, 1951.

47. Douglas, W. W. The effect of hexamethonium on carotid body responses in the cat. J. Physiol., 115: 70 p. 1951.

48. Douglas, W. W. and Gray, J. A. B. Unpublished.

49. Douglas, W. W. and Matthews, P. B. C. Acute tetraethylphosphate poisoning in cats and its modifications by atropine or hyoscine. J. Physiol., 116: 202-218, 1952.

50. Douglas, W. W. and Paton, W. D. M. The mode of action of tetraethylpyrophosphate at the cat's neuromuscular junction. J. Physiol., **115**: 71–72 p.

51. Douthwaite, A. H. and Thorne, M. G. The effects of hexamethonium bromide on the stomach. Brit. M. J., **1**: 111–114, 1951.

52. Ellerker, A. R. Decamethonium iodide as a curarizing agent in general anaesthesia. Brit. M. J., **2**: 398–399, 1950.

53. Emmelin, N. Ett syntetiskt amne med kurareverkan for kliniskt bruk. Nord. Med., **43**: 255–261, 1950.

54. Enderby, G. E. H. Controlled circulation with hypotensive drugs and posture to reduce bleeding in surgery. Lancet, **1**: 1145–1147, 1950.

55. Enderby, G. E. H., Armstrong Davison, M. H., Boyes, Korkis, F., Barnes, M., Shackleton, R. P. W., Scurr, C. F., Gillies, J., Musgrove, A. H. and Wyman, J. B. Discussion on the use of hypotensive drugs in surgery. Proc. Roy. Soc. Med., **44**: 829–840, 1951.

56. Enderby, G. E. H. and Pelmore, J. F. Controlled hypotension and postural ischaemia to reduce bleeding in surgery. Lancet. **1**: 663-666, 1951.

57. Feldberg, W. Effects of ganglion-blocking substances on the small intestine. J. Physiol., **113**: 483–505, 1951.

57a. Feldberg, W., and Toh, C. C. Unpublished.

58. Finnerty, F. A. and Freis, E. D. Experimental and clinical evaluation in man of hexamethonium (C6), a new ganglionic blocking agent. Circulation, **2**: 828–836, 1950.

59. Finnerty, F. A. and Freis, E. D. Clinical appraisal of hexamethonium (C6) in peripheral vascular diseases. New England J. Med., **245**: 325–328, 1951.

60. Fleckenstein, A., Hille, H. A. and Adam, W. L. Aufhebung der Contractur-Wirkung depolarisierender Katelektrotonica durch Depolarisation im Anelektrotonus. Pflügers Arch., ges. Physiol., **2 53**: 264, 1951.

61. Frankel, E. Methonium compounds in hypertension. Lancet, **1**: 408–409, 1951.

62. Freeman, Z. Hexamethonium in hypertension. Brit. M. J., **2**: 1496, 1950.

63. Freeman, Z. Unexpected failure of hexamethonium explained. Brit. M. J., **1**: 1079, 1951.

64. Freis, E. D. Methonium compounds in hypertension. Lancet, **1**: 909, 1951.

65. Freis, E. D. Veratrum viride and hexamethonium in the treatment of severe diastolic hypertension. M. Ann. District of Columbia, **20**: 297–304, 1951.

66. Freis, E. D., Stanton, J. R., Finnerty, F. A., Schnaper, H. W., Johnson, R. L., Rath, C. E. and Wilkins, R. W. The collapse produced by venous congestion of the extremities or by venesection following certain hypotensive agents. J. Clin. Investigation, **30**: 435–444, 1951.

67. Ginzel, K. H., Klupp, H. and Werner, G. Zur Pharmakologie von, α-ω-bis-Quarternaeren Ammoniumverbindungen. II. Mitteilung: Vergleichende Untersuchungen ueber einige aliphatishe Dicarbonsaeureester. Arch. internat. de pharmacodyn. et de thérap., **87**: 79–98, 1951.

68. Ginzel, K. H., Klupp, H. and Werner, G. Methoden zur biologischen Wertbestimmung und Charakterisierung von neuromuskulaer laehmenden Stoffen. Sonderdruck aus der "Scientia Pharmaceutica." Jahrung 19, Seite 164–176, 1951.

69. Ginzel, K. H., Klupp, H. and Werner, G. Ueber nikotinartige Wirkungen auf die Skelettmuskulatur. Separatum Experientia, Verlag Birkhaeuser, Basel/Schweiz, Vol. VII/10, 1951, p. 387.

70. Ginzel, K. H., Klupp, H. and Werner, G. Zur Pharmakologie von α-ω-bis Quarternaeren Ammoniumverbindungen. I. Mitteilung: Neuromusculaere und ganglionaere Wirkungen des Adipinsaeure-bis-Cholinesters. Arch. internat. de pharmacodyn. et de thérap., **86**: 385–406, 1951.

71. Ginzel, K. H., Klupp, H. and Werner, G. Die Wirkung einiger aliphatischer α-ω-bis-quarternaerer Ammonium Verbindungen auf die Skelettmuskulatur. Arch. exper. Path. u. Pharmakol., **213**: 453–466, 1951.

72. Glajchen, D. Hexamethonium in acute ventricular failure. Brit. M. J., **2**: 797, 1951.

73. Graham, J. G. and Campbell, A. Absorption of hexamethonium. Brit. M. J., **1**: 1514–1515, 1951.

74. Gray, A. J. Decamethonium iodide as a muscle relaxant in abdominal surgery. Lancet, **1**: 253–254, 1950.

75. Grob, D. and Harvey, A. M. Observations on the effects of the autonomic blocking agent, bis-trimethylammonium pentane dibromide (C5) in normal subjects and in patients with peripheral vascular disease and hypertension, and comparison with tetraethylammonium chloride. Bull. Johns Hopkins Hosp., **87**: 616–639, 1950.

76. Grob, D., Holaday, A. and Harvey, A. McG. The effects of bis-trimethylammonium decane diiodide and dibromide on neuromuscular function and on induced convulsions in man. New England J. Med., **241**: 812–815, 1949.

77. Guild, A. A. Decamethonium iodide in muscular hypertonus. Lancet, **2**: 251–252, 1950.

78. Guiot, G., Damoiseau, P. and Poloukhine, N. Ganglioplegiques et chirurgie cerebrale. Anesth. & Analg., **8**: 1–6, 1951.

79. Gutton, A. C. and Reeder, R. C. Quantitative studies on the autonomic actions of curare. J. Pharmacol. & Exper. Therap., **98**: 188,–193 1950.

80. Harper, J. K. Pentamethonium in surgical emergencies. Brit. M. J., **1**: 1262–1263, 1951.

81. Harington, M. Unpublished.

82. Harris, L. C. and Dripps, R. D. The use of decamethonium bromide for the production of muscular relaxation. Anesthesiology, **11**: 215–223, 1950.

83. Hewer, A. J. H., Lucas, B. G. B., Prescott, F. and Rowbotham, E. S. Decamethonium iodide as a muscle relaxant in anaesthesia. Lancet, **1**: 817–819, 1949.

84. Hillis, B. R. and Kelly, J. C. C. Hiccup and hexamethonium. Brit. M. J., **1**: 1143, 1951.

85. Hillis, B. R. and Kelly, J. C. C. Effect of hexamethonium iodide on lobeline-stimulated coughing Glasgow M. J., **32**: 72–76, 1951.

86. Hirson, C. and Kelsall, A. R. Paralytic ileus after hexamethonium. Brit. M. J., **1**: 1332, 1951.

87. Hobson, J. A. and Prescott, F. Comparison of decamethonium iodide with d-tubocurarine in controlling electrically induced convulsions. Lancet, 1: 819–820, 1949.

88. Holaday, D. A., Harvey, A. M. and Grob, D. The use of bis-trimethylammonium decane dibromide in anesthesia. New England J. Med., 241: 816–819, 1949.

89. Holt, M. C. and Litchfield, J. W. Bromism on low-salt diets. Lancet, 1: 347, 1951.

90. Holton, Pamela and Ing, H. R. The specificity of the trimethylammonium group in acetylcholine. Brit. J. Pharmacol., 4: 190–196, 1949.

91. Hughes, G. Use of Pentamethonium and posture to reduce bleeding in fenestration operations. Lancet, 1: 666–667, 1951.

92. Hunter, A. R. Decamethonium and hexamethonium. A clinical and experimental study. Brit. J. Anaesth., 22: 218–234, 1950.

93. Hunter, A. R. Hexamethonium bromide. Lancet, 1: 251–252, 1950.

94. Hutter, O. F. and Pascoe, J. E. Decurarization by decamethonium. Brit. J. Pharmacol, 6: 691–695, 1951.

95. Jarcho, L. W., Eyzaguirre, C., Talbot, S. A. and Lilienthal, J. L., Jr. Neuromuscular excitation: responses of normal and denervated mammalian muscle to *bis*-trimethylammonium decane (C10) and to d-tubocurarine. Am. J. Physiol., 162: 475–488, 1950.

96. Jarcho, L. W., Berman, B., Eyzaguirre, C. and Lilienthal, J. L., Jr. Curarization of denervated muscle. Ann. New York Acad. Sc., 54: 337–346, 1951.

97. Kay, A. W. and Smith, A. N. Effect of hexamethonium iodide on gastric secretion and motility. Brit. M. J., 1: 460–463, 1950.

98. Kay, A. W. and Smith, A. N. Effect of oral hexamethonium salts on gastric secretion. Brit. M. J., 2: 807–809, 1950.

99. Kay, A. W. and Smith, A. N. The effect of the ganglion-blocking methonium salts on gastric secretion and motility. Gastroenterology, 18: 503–517, 1951.

100. Keir, R. McD. S. Case of tetanus treated with decamethonium iodide. Brit. M. J., 2: 984–985, 1950.

101. Kensler, C. J. The anticurare effect of tetraethylammonium ion in the cat. Brit. J. Pharmacol., 5: 204–209, 1950.

102. Kilpatrick, J. A. and Smirk, F. H. Comparison of oral and subcutaneous administration of methonium salts in the treatment of high blood-pressure. Lancet, 1: 8–12, 1952.

103 King, H. Curare Alkaloids. Part X. Some alkaloids of *Strychnos toxifera*. Rob. Schomb. J. Chem. Soc., p. 3263–3271, 1949.

104. Laurence, D. R. and Stacey, R. S. The effect of methonium compounds on nicotine convulsions. Brit. J Pharmacol., 7: 70–84, 1952.

105. Laurence, D. R. and Stacey, R. S. Hypoglycaemia masked by methonium. Lancet, 2: 1145, 1951.

106. Lewis, I. Value of hypotensive drugs in minimising blood-loss in thoracic surgery. Lancet, 2: 150–151, 1951.

107. Locket, S., Swann, P. G. and Grieve, W. S. M. Methonium compounds in the treatment of hypertension Brit. M J., 1: 778–783, 1951.

108. Locket, S. Paralytic ileus after hexamethonium. Brit. M. J., 1: 1331–1332, 1951.

109. Locket, S., Swann, P. G., Grieve, W. S. M. and Player, H. P. M. & B. 1863, a homologue of hexamethonium, in treatment of hypertension. Brit. M. J., 1: 254–258, 1952.

110. Loewe, S. and Harvey, S. C. Equidistance concept and structure-activity relationship of curarizing drugs Arch. f. exper. Path. u. Pharmakol., 214: 214–226, 1952.

111. Lyons, W. G. and Lord, P. J. Paralytic ileus after hexamethonium. Brit. M. J., 2: 176, 1951.

112. MacFarlane, D. W., Unna, K. R., Pelikan, E. W., Cazort, R. J., Sadove, M. S. and Nelson, J. T. Evaluation of curarizing drugs in man. III. Antagonism to curarizing effect of d-tubocurarine and decamethylene-*bis* (trimethylammonium bromide). J. Pharmacol. & Exper. Therap., 99: 226–233, 1950.

113. McGill, R. J. Hiccup and hexamethonium. Brit. M. J., 1: 948, 1951.

114. MacIntyre, A. R. and King, R. E. Contraction of denervated muscle produced by d-tubocurarine. Science, 97: 516, 1943.

115. Mackey, W. A. and Shaw, G. B. Paralytic ileus after hexamethonium. Brit. M. J., 1: 1205, 1951.

116. Mackey, W. A. and Shaw, G. B. Oral hexamethonium bromide in essential hypertension. Brit. M. J., 2- 259–265, 1951.

117. Margolis, L. H., Simon, A. and Bowman, K. M. Effects of decamethonium bromide (C10) and d-tubocurarine on electro-convulsions. Arch. Neurol. & Psychiat., 65: 174–180, 1951.

118. Masini, V. and Rossi, P. Methonium compound in absorption delaying solution in therapy of arterial hypertension. Riforma Medica, Naples, 65: 898–899, 1951. Cited J. Am. M. A., 148: 413, 1952.

119. Miles, B. E., de Wardener, H. E., Churchill-Davidson, H. C., and Wylie, W. D. The effect on the renal circulation of pentamethonium bromide during anaesthesia. Clin. Sci., 11: 73–79, 1952.

120. Milne, G. E. and Oleesky, S. Excretion of the methonium compounds. Lancet, 1: 889–890, 1951.

120a. Milne, G. E. and Oleesky, S. Absorption of hexamethonium. Brit. M. J., 2: 177, 1951.

121. Morrison, B. and Paton, W. D. M. Unpublished.

122. Murphy, E. A. Treatment of hypertension with hexamethonium bromide. Lancet, 2: 899–901, 1951.

123. Organe, G. Decamethonium iodide (bistrimethylammonium decane diiodide) in anaesthesia. Lancet, 1: 773–774, 1949.

124. Organe, G., Paton, W. D. M. and Zaimis, E. J. Preliminary trials of bistrimethylammonium decane and pentane diiodide (C10 and C5) in man. Lancet, 1: 21–23, 1949.

125. Paradis, B. Nouveau curarisant de synthese. Laval méd., 15: 761–768, 1950.

126. Paton, W. D. M. The pharmacology of curare and curarising substances. J. Pharm. & Pharmacol., 1: 273–286, 1949.

127. Paton, W. D. M. Paralysis of autonomic ganglia and the therapeutic effects of ganglion-blocking drugs. Brit. M. J., **1:** 773–778, 1951.

128. Paton, W. D. M. The pharmacology of decamethonium. Ann. New York Acad. Sc., **54:** 347–361, 1951.

129 Paton, W. D. M. Unpublished.

130. Paton, W. D. M. and Perry, W. L. M. Depolarization and transmission block in the cat's superior cervical ganglion. J. Physiol., **112:** 49 P., 1951.

131. Paton, W. D. M. and Perry, W. L. M. The pharmacology of the toxiferines. Brit. J. Pharmacol., **6:** 299–310, 1951.

132. Paton, W. D. M. and Perry, W. L. M. The relationship between depolarization and the action potential complex of the cat's superior cervical ganglion. J. Physiol., **114:** 47 P, 1951.

132a. Paton, W. D. M. and Perry, W. L. M. Unpublished.

133. Paton, W. D. M. and Vianna Dias, M. Unpublished.

134. Paton, W. D. M. and Walker, J. Methonium compounds in hypertension. Lancet, **1:** 473–474, 1951.

135. Paton, W. D. M. and Zaimis, E. J. Curare-like action of polymethylene *bis*-quaternary ammonium salts. Nature, **161:** 718–719, 1948.

136. Paton, W. D. M. and Zaimis, E. J. Clinical potentialities of certain bisquaternary salts causing neuromuscular and ganglionic block. Nature, **162:** 810, 1948.

137. Paton, W. D. M. and Zaimis, E. J. The pharmacological actions of polymethylene bistrimethylammonium salts. Brit. J. Pharmacol., **4:** 381–400, 1949.

138. Paton, W. D. M. and Zaimis, E. J. The action of curarizing substances on respiration in the cat. J. Physiol., **108:** 34 P., 1949.

139. Paton, W. D. M. and Zaimis, E. J. The properties of polymethylene bistrimethylammonium salts. J. Physiol., **108:** 55 P., 560, 1949.

140. Paton, W. D. M. and Zaimis, E. J. Actions and clinical assessment of drugs which produce neuromuscular block. Lancet, **2:** 568–570, 1950.

141. Paton, W. D. M. and Zaimis, E. J. The action of d-tubocurarine and of decamethonium on respiratory and other muscles in the cat. J. Physiol., **112:** 311–331, 1951.

142. Paton, W. D. M. and Zaimis, E. J. Paralysis of autonomic ganglia by methonium salts. Brit. J. Pharmacol., **6:** 155–168, 1951.

143. Pelikan, E. W., Unna, M. R., MacFarlane, D. W., Cazort, R. J., Sadove, M. S. and Nelson, J. T. Evaluation of curarizing drugs in man. II. Analysis of response curves and effects of repeated administration of d-tubocurarine, dimethyl-d-tubocurarine and decamethylene-bis(trimethylammonium bromide). J. Pharmacol. & Exper. Therap., **99:** 215–225, 1950.

144. Penny, C. J. and Shackleton, R. P. W. A case of eclampsia treated with hexamethonium bromide. Lancet, **2:** 617–618, 1951.

145. Philippot, E. and Dallemagne, M. J. Synergies et antagonismes a la junction neuro-musculaire. Action de sels d'alkyltrimethyl-ammonium. Arch. internat. de physiol., **59:** 357–373, 1951.

146. Restall, P. A. and Smirk, F. H. The treatment of high blood pressure with hexamethonium iodide. New Zealand M. J., 206–209, 1950.

147. Restall, P. A. and Smirk, F. H. Variation in blood pressure induced by hexamethonium bromide in conjunction with positive and negative pressures applied to the body surface. Proc Univ. Otago. Med. Sch., **29:** 10–11, 1951.

148. Rollason, W. N. Reduction of surgical haemorrhage. Brit. M. J., **1:** 1447, 1951.

149. Rose, J. C. and Wemple, J. N. Hexamethonium (C6) in the management of causalgia. U. S. Armed Forces M. J., **2:** 937, 1951.

150. Rosenheim, M. L. Bromism on low-salt diets (Letter). Lancet, **1:** 347, 1951.

150a. Rosenheim, M. L. Medical treatment & hypertension (Proc. Roy. Soc. Med.). Lancet, **1:** 492–493, 1952.

151. Riddell, M. J. Interdigestive gastric secretion in duodenal ulcer. Brit. M. J., **2:** 1498, 1951.

151a. Riker, W. F. and Wescoe, W. C. The Pharmacology of Flaxedil. Ann. New York Acad. Sc., **54:** 373–392, 1951.

152. Saunders, J. W. Negative pressure device for controlled hypotension during surgical operations. Lancet, ii, p. 43, 1952.

152a. Saville, S. Pentamethonium in hypertension. Lancet, **2:** 358–360, 1950.

153. Schachter, M. Hexamethonium and insulin hypoglycaemia. J. Physiol., **115:** 206–209, 1951.

154. Schnaper, H. W., Johnson, R. L., Tuohy, E. B. and Freis, E. D. The effect of hexamethonium as compared to procaine or metycaine lumbar block on the blood flow to foot of normal subjects. J. Clin. Investigation, **30:** 786–791, 1951.

155. Scott, L. D. W., Kay, A. W., O'Hare, M. M. and Simpson, J. A. Hexamethonium in duodenal ulcer. Brit. M. J., **2:** 1470–1472, 1950.

156. Scurr, C. F. Decamethonium iodide. Lancet, **1:** 842, 1949.

157. Sellick, B. A. Decamethonium iodide in myasthenia gravis. Lancet, **2:** 822, 1950.

158. Shackleton, R. P. W. Reduction of surgical haemorrhage Brit. M. J., **1:** 1054–1056, 1951.

159. Smirk, F. H. Methonium compounds in hypertension. Lancet, **2:** 477, 1950.

160. Smirk, F. H. Practical details of the methonium treatment of high blood pressure. New Zealand M. J., **49:** 637–643, 1950.

161. Smirk, F. H. Methonium compounds in high blood pressure. Lancet, **1:** 346–347, 1951.

162. Smirk, F. H. and Alstad, K. S. Treatment of arterial hypertension by penta- and hexamethonium salts. Based on 150 tests on hypertensives of varied aetiology and 53 patients treated for periods of two to fourteen months. Brit. Med. J., **1:** 1217–1228, 1951.

163. Somerville, J. and Allan, J. A case of drug eruption induced by hexamethonium bromide. Brit. M. J., 1: 864–865, 1951.
164. Spencer, C. H. and Coakley, C. S. Clinical impressions of decamethonium bromide (C10) in anesthesia. M. Ann. District of Columbia, 19: 132–136, 1950.
165. Taylor, E. P. and Collier, H. O. J. Synthetic curarizing agents structurally related to d-O.O.-dimethyltubocurarine. Nature, 167: 692, 1951.
166. Thomas, K. B. Decamethonium iodide in anaesthesia. Lancet, 1: 936, 1949.
167. Thomas, O. M. and Williams, R. G. Paralytic ileus after hexamethonium. Brit. M. J., 1: 1331, 1951.
168. Turner, R. "Medical sympathectomy" in hypertension: the clinical study of methonium compounds. Lancet, 2: 353–358, 1950.
169. Turner, R. W. D. Current therapeutics XLVII.—The methonium compounds. Practitioner, 167: 541–553, 1951.
170. Turner, R. Methonium compounds in hypertension. Lancet, 1: 408, 1951.
171. Unna, K. R., Pelikan, E. W., MacFarlane, D. W., Cazort, R. J., Sadove, N. S., Nelson, J. T. and Drucker, A. P. Evaluation of curarizing drugs in man. I. Potency, duration of action and effects on vital capacity of d-tubocurarine, dimethyl-d-tubocurarine and decamethylene bis (trimethylammonium bromide). J. Pharmacol. & Exper. Therap., 98: 318–329, 1950.
172. Unna, K. R., Pelikan, E. W., MacFarlane, D. W., Cazort, R. J., Sadove, M. S. and Nelson, J. T. Evaluation of curarizing agents in man. J. Am. M. A., 144: 448–451, 1950.
173. Unna, K. R., and Pelikan, E. W. Evaluation of curarizing drugs in man. VI. Critique of experiments on unanaesthetised subjects. An. New York Acad. Sc., 54: 480–490, 1951.
174. Vetten, K. B. and Nicholson, J. C. A comparison of decamethonium iodide and d-tubocurarine chloride in anaesthesia. Anaesthesia, 5: 175–186, 1950.
175. Volpitto, P. P. Experiences with ultra-short acting intravenous barbiturates combined with decamethonium bromide for endotracheal intubation. Anesthesiology, 12: 648–655, 1951.
176. Vourc'h, G. Sur trois cas d'hypotension controlée par hexaméthonium en neuro-chirurgerie. Gazette Médicale, 59: 163–166, 1952.
176a. Walker, J. Some new curarising substances. J. Chem. Soc. p. 193. 1950.
177. Werkö, L., Frisk, A. R., Wade, G. and Eliasch, H. Effect of hexamethonium bromide in arterial hypertension. Lancet, 2: 470, 1951.
178. Wien, R. and Mason, D. F. J. Some actions of hexamethonium and certain homologues. Brit. J. Pharmacol., 6: 611–629, 1951.
179. Wylie, W. D. and Churchill-Davidson, H. C. Side action of hexamethonium compounds (Letter). Brit. M. J., 2: 54, 1951.
180. Young, I. M. Abdominal relaxation with decamethonium iodide (C10) during caesarean section. Lancet, 1: 1052, 1949.
181. Young, I. M. The action of decamethonium iodide (C10) on foetal neuro-muscular transmission and its transfer across the placenta. J. Physiol., 109: 31 P., 1949.
182. Young, I. M. The placental transfer of hexamethonium bromide in the rabbit and its appearance in the amniotic fluid. J. Physiol., 116: 4–5 P., 1951.
183. Young, I. M., De Wardener, H. E. and Miles, B. E. Mechanism of the renal excretion of methonium compounds. Brit. M. J., 2: 1500–1501, 1951.
184. Zaimis, E. J. Some effects of decamethonium iodide on skeletal muscle. J. Physiol., 110: 10 P., 1949.
185. Zaimis, E. J. The synthesis of methonium compounds, their isolation from urine, and their photometric determination. Brit. J. Pharmacol., 5: 424–430, 1950.
186. Zaimis, E. J. The action of decamethonium on normal and denervated mammalian muscle. J. Physiol., 112: 176–190, 1951.
187. Zaimis, E. J. Unpublished.

ANTIDIURETIC SUBSTANCES

MARY PICKFORD

Physiology Department, University of Edinburgh

TABLE OF CONTENTS

Detection and Assay 254
1. Substances Acting Directly on the Cells of the Renal Tubules (Antidiuretic hormone of the posterior lobe of the pituitary gland.) 256
   The effect of ADH on the absorption of water from the alimentary canal 256
   The effect of ADH on the ureters 256
   The effect of ADH on other extrarenal sites 256
   Site of action of ADH in the kidney 257
   The role of the renal nerves in the action of ADH 258
   The action of ADH on the tubules 258
   Concentration of posterior lobe extract necessary to produce antidiuresis 260
   The rate of ADH release from the posterior lobe 260
   The disappearance of ADH from the blood 261
   Form and composition of ADH as released from the posterior lobe 261
2. Substances Whose Site of Action is in the Central Nervous System 262
   Acetylcholine 263
   Adrenaline 264
   Oxygen lack and carbon dioxide excess 264
3. Substances Affecting the Circulation of Blood through the Kidney 265
   Adrenaline and noradrenaline 266
   Histamine 267
   Adenylic acid derivatives 268
   Renin and hypertensin 268
   Vasopressin, other vasoactive substances, and the intrarenal redistribution of blood 269
4. Miscellaneous Antidiuretic Substances and Causes of Antidiuresis 271
   Ferritin 271
   Hepato-renal syndrome 271
   Adrenal cortical disorders 272
   Antidiuretic substances found in pregnancy 273
   Various steroids 274
5. Summary 275

In this review the discussion will be limited to a consideration of those antidiuretic substances which are found in animal tissues and body fluids. The qualification antidiuretic, as used here, relates to those substances which cause water retention either by an action in the kidney or on other tissues. For the purpose of discussion, antidiuretic substances have been classified in four groups as shown in the table of contents. For the sake of brevity the letters ADH have been used in some places where "posterior pituitary extract containing vasopressor and antidiuretic fractions" would have been more accurate.

Detection and Assay

Since reliable methods of detection and assay are essential for the identification and proper understanding of the action of antidiuretic substances, a short account follows of the methods available. So far, owing to our ignorance of the nature of most antidiuretic substances, biological assay is necessary. All the methods depend on testing tissue extracts or body fluids from one animal on a second test animal(s) which is in a state of water diuresis. Most often the method of Burn (23), or a modification of it (80), has been used. In this method observation is made of the time taken for a group of rats to excrete an orally given volume of water with or without the subcutaneous injection of the material to be tested. The unknown substance can also be administered intraperitoneally (12). Instead of rats, mice have been used (166). It seems, however, that mice are not wholly satisfactory, since their control rates of water excretion are less regularly reproducible than those of rats. Bigger animals such as rabbits and dogs have also been used, and have the advantage that both standard and unknown can be compared on the same animal, and that fewer animals are necessary (16, 36, 95, 143, 170, 173, 246). Recently, Jeffers, Livezey and Austin (121) have described a sensitive rat method in which the substances to be tested are given intravenously during ethanol narcosis, and in which standard and unknown can be used on the same animal during the course of a single set of observations. Man, too, has been used as a test animal (18). The sensitivity of the tests varies greatly. The rat method of Burn detects about 20 mU. of antidiuretic activity, that of Jeffers, Livezey and Austin 0.02 mU. In the rabbit 0.2 mU. can be detected (246), and in the dog 0.1 mU. (170). The methods mentioned were specifically designed to measure the activity of the antidiuretic hormone of the posterior lobe of the pituitary (ADH). Occasionally discrepancies have appeared that can be accounted for only by assuming that antidiuretic substances are made in tissues other than the posterior lobe (7, 246), or that the test method was unsuitable. At least in a number of instances the latter was true. Body fluids may contain substances which, on subcutaneous injection, "augment" the action of antidiuretic hormone by stabilising it, or causing it to be more slowly absorbed (167). Sometimes, too, subcutaneously or intraperitoneally injected material causes pain, and both pain and excitement, as will be shown later, may lead to antidiuresis (239). As an example of the confusion that may arise from the use of unsuitable test methods may be cited the finding that rat serum obtained by heart puncture was more actively antidiuretic than serum collected from the carotid artery (81). The sera were tested on rats and administered subcutaneously. Further difficulties of interpretation arose from the finding that carotid plasma was without antidiuretic effect, and that there appeared to be two antidiuretic substances, one stable and the other unstable (81). Ames and van Dyke (4), using the method of Jeffers, Livezey and Austin, *i.e.*, intravenous administration of the test substance, obtained consistent results that were easy to explain. They could find no ADH in rat blood collected by decapitation provided that the animals were tranquil until the moment of death, but could always detect ADH in serum collected by heart puncture or under ether anaesthesia, even if the rats had first been hydrated. The ADH concentration was identical in serum and heparinised plasma. Under no conditions of blood collection could ADH be detected if the rats had first been hypophysectomised. Thus it is evident that great care must be taken to control the circumstances in which body fluids are collected, and, as far as possible, the material to be tested should be injected intravenously (49) rather than subcutaneously or intraperitoneally, since it is then less likely to evoke secretion from the test rat's own posterior lobe.

1. SUBSTANCES ACTING DIRECTLY ON THE CELLS OF THE RENAL TUBULES

There is one tissue substance that is now generally believed to be antidiuretic by virtue of a specific effect on the cells of the renal tubules, namely, the antidiuretic hormone of the posterior lobe of the pituitary. The evidence for the site of origin of ADH, that it may indeed be considered a hormone, and that it is antidiuretic and not diuretic as was thought at first, has been given in previous reviews (67, 97, 169, 178, 240), and therefore will not be repeated.

There have been suggestions that ADH has mainly an extrarenal action. Two of these suggestions can quickly be discarded.

The effect of ADH on the absorption of water from the alimentary canal

Several workers have shown that posterior pituitary extract does not prolong the emptying time of the stomach (26, 39, 105, 186), nor delay absorption from the intestine. Thus, delayed absorption cannot be considered a factor in the antidiuretic effect of ADH.

The effect of ADH on the ureters

That the ureters go into spasm after posterior pituitary extract administration has been put forward as an explanation of its antidiuretic effect (148, 150). This may, in part, account for the action if fairly large doses of extract are given, but is certainly not the whole or most important reason. Ross and Stehle (192) could find no spasm, and antidiuresis always occurred in the experiments of Verney (238) on the isolated kidney, and of Klisiecki, Pickford, Rotschild and Verney (126), and of Pickford (176) on the conscious dog, in which a cannula had been inserted whose tip lay in the pelvis of the kidney, thus eliminating all possibility of ureteral obstruction to the flow of urine.

The effect of ADH on other extrarenal sites

Molitor and Pick are the chief protagonists of the view that ADH acts extrarenally. They and their co-workers published a series of papers in which they believed they had shown that posterior lobe extract acts either on a water control centre in the central nervous system, or on the water-binding capacity of the tissues generally (161–164). Theobald (222) could not repeat, and criticised their work, and Janssen (120) showed by means of spinal cord and nerve sections that the antidiuretic action of posterior lobe extract does not depend on the integrity of the central nervous system. Buschke (26), following a different line of investigation, found that in nephrectomised rabbits the administration of posterior pituitary extract raised the blood chloride concentration. He concluded, therefore, that posterior pituitary hormone acts on the tissues to release chloride, and that this, in turn, affects the excretion of water. There is no evidence that with moderate water loading physiological amounts of ADH cause tissue hydration. On the contrary, v. Konschegg and Schuster (129) were the first amongst many to show that during antidiuresis posterior pituitary extract increased the water content of the blood. This observation has been confirmed in various animals, including man (15, 39, 190, 237). In dogs, for instance, pituitary antidiuresis may give rise to marked salivation (222). Thus, the presentation of insufficient water to the kidneys cannot be the cause of pituitary antidiuresis. There is further evidence of direct renal action in that the polyuria of the isolated perfused kidney is lessened if the kidney is supplied with posterior pituitary extract (210). This point was strikingly shown by Verney (238), who included a head in the perfusion circuit. Provided that the pituitary gland was present in the head the polyuria of the isolated kidney was reduced, but if the gland had been removed the polyuria persisted. The weight of evidence is all in favour of the antidiuretic effect of posterior pituitary extract being a direct renal one. To say this is not to exclude the possibility that extracts may in addition, and under some circumstances, have an action, perhaps nonspecific on the tissues (149, 213, 232, 259).

Site of action of ADH in the kidney

Accepting that the antidiuretic action of ADH is renal, it is then necessary to seek for the exact site of action in the kidney. The site certainly varies with the type of animal observed. Below the order Reptilia posterior pituitary extract is not antidiuretic. In reptiles, using alligators, Burgess, Harvey and Marshall (21) found that pituitary antidiuresis was accompanied by a fall in glomerular filtration rate (GFR), with no suggestion of increased water reabsorption in the tubules. In chickens the same observers found that antidiuresis could be induced with only a small change in GFR, though with larger doses of pitressin the filtration rate was markedly depressed. For rabbit, rat, dog and man there is a mass of evidence, both direct and indirect, that antidiuresis occurs without change in the vascular relations in the kidney. Direct measurements of renal blood flow have been made by means of the Rein thermostromuhr, and no change from normal was found as the result of administering ADH to conscious dogs (94, 244). Walker, Schmidt, Elsom and Johnston (248), observing the effect of ADH in both dogs and rabbits, found no parallelism between renal blood flow and urine flow, and concluded that the changes they observed in the latter depended on alterations in the activity of the tubule cells. Indirect evidence has been obtained by means of renal clearance measurements, and has given no cause to believe that pituitary antidiuresis is necessarily associated with changes in GFR or renal plasma flow (RPF) (199, 206). Lamport (138), by calculations based on his own results and those of others, found that in pituitary antidiuresis there was no consistent change in intracapillary pressure or effective arteriolar resistance in the kidney of the dog. A few observers have tried to obtain information about the action of ADH by measuring renal oxygen consumption under various conditions. The earlier work is difficult to interpret as it was done on isolated kidneys or anaesthetized eviscerated animals (65, 127). More recently, Cutting and McCance (42, 43) observed the metabolic rate of kidney slices from adult and infant rats and noted that posterior pituitary extract increased oxygen consumption of adult kidney, but was almost without action on infant kidney slices. If we allow that ADH has an action on the kidney and causes it to do work, these facts correlate well with the known ineffectiveness of ADH as an antidiuretic in young infants.

It may safely be concluded that in mammals, when ADH is used in physiological amounts, its effect is due to a specific action on the tubule cells of the renal epithelium.

The role of the renal nerves in the action of ADH

Motzfeldt (165) believed that the antidiuretic action of posterior pituitary extract was renal in origin, and that the sympathetic nervous system played some part in the reaction. He made his observations on anaesthetized rabbits. Later observers, using unanaesthetized animals, have not been able to show that the peripheral sympathetic nerves are in any way concerned in the action of ADH (64, 126, 194, 223). For example, Samaan (194) made his observations on conscious dogs whose two kidneys had been separately cannulated, and one of which had been denervated by stripping the walls of the artery and vein and separating the kidney from all its attachments except the main blood vessels and ureter. In this well controlled experiment he could find no difference in the response of the two kidneys to ADH.

The action of ADH on the tubules

Burgess, Harvey and Marshall (21) pointed out that birds are the lowest of the vertebrate series to respond to ADH by a reduction in the rate of urine flow without the necessary accompaniment of a marked reduction in GFR, and that they were also the first animals to show a thin segment in the loop of Henle. These authors therefore made the interesting suggestion that the thin segment is the site both of water reabsorption and of the action of ADH. As the thin segment looks admirably adapted to the transfer of water these suggestions were accepted and are often quoted as though both hypotheses were proven. Further, it was noticed that in certain desert rodents the thin segment is longer than in related species living near ample water supplies (128, 208). The idea of Burgess *et al.* is attractive, but should not be allowed to close the mind against other possibilities. With regard to the observations on birds, a close examination of the evidence shows in how many ways the kidney of the bird differs from that of dog and man. In the first place, relatively large amounts of ADH have to be used to produce antidiuresis, the GFR is extremely variable, and there is a distinct tendency for it to rise during water diuresis and to fall during hormonal antidiuresis. Further, the onset of diuresis after the administration of water by stomach tube may show the typical mammalian latency (126) between the peak of water load and the peak of diuresis, or there may be no latency at all. Again, ADH reduces the rate of urine flow even in dehydrated chickens excreting an already concentrated urine (130). Finally, the thin segment of Henle's loop is present to a variable extent in different birds and is always short (114). It has to be remembered, too, that birds and mammals are separately developed branches of the vertebrate tree and that hormones common to both may not have been put to the same use, as has happened with oxytocin in the Amphibia (102, 211). Thus, in birds, the thin segment may well be concerned with the reabsorption of water, but that is not to say that it is also the site of action of ADH. Deductions made from observations on rats can perhaps be more easily transferred to other mammals; but, again, rodents are in many ways highly specialised. Ames and van Dyke (3) found that the kangaroo rat, an inhabitant of desert country, had relatively more ADH in its pituitary gland than the common laboratory rat, and excreted absolutely more ADH into its urine. Thus, desert rats may have an increased supply of ADH and longer thin segments for it to act on (128, 208), or they may have a double and unrelated protection against water loss. That such animals do not rely on their kidneys alone for water conservation was shown by Schmidt-Nielsen and Schmidt-Nielsen (197), who found that they lose only half as much water through the lungs as do most rodents.

Although in the thin segment the cells are flat and have an inactive appearance this does not, of itself, prove that they have no power to do work. It has been shown that the epithelial cells in the gills of eels and some other teleosts are able to transfer water and chloride from blood to seawater against a high osmotic pressure and, moreover, that the chloride and water are conveyed independently of each other (125, 196). However, the working cells appear to be large ones with central nuclei at the base of the leaflets, and are unlike the flat respiratory epithelium (125a). There is here, then, not even an indirect argument for the hypothesis that the thin segment of the loop of Henle does secretory work. In general, the cuboidal cells of the distal tubules appear much more likely to take an active part in the formation of the final urine.

Wirz, Hargitay and Kuhn (256) looked for the site of urine concentration by means of cryoscopic observations on kidney slices from rats. They found that the osmotic pressure of the kidney rose steadily from the outer border of the cortex to the tip of the papilla. They interpreted this as meaning that tubular urine was concentrated in the descending limb, diluted again in the ascending, and finally concentrated in the collecting duct. These findings suggest the importance of two parts of the tubules in the concentration of urine. This work is, however, open to the criticism that diffusion may have occurred from, say, the highly concentrated urine in the collecting ducts to the relatively less concentrated solution in the loops of Henle. This point may be settled by work now in progress on golden hamsters, in which the papilla protrudes into the hilus of the ureter, so that both capillary blood and urine from specific tubules can be collected from that part of the kidney (Wirz, personal communication).

Smith (207) discusses the results of some observations (252) on the response of dogs to osmotic diuresis with and without pitressin infusion, and shows that they could be interpreted as indicating that the function of the thin segment is the passive reabsorption of water so that the distal limb receives urine isosmotic with the plasma, though not of the same composition, many constituents having been absorbed in the proximal tubule (248). The distal tubule is, then, responsible for the final adjustment of urine composition by the reabsorption of Na and water, its activity being hormonally controlled. In the case of water the hormone concerned would be ADH.

There is yet another possibility; Ljungberg (146) by means of a 3 mm.-diameter punch collected radial cylinders from the kidneys of rabbits and examined them in serial section for their Cl content at various levels from the surface. After making allowance for the fact that all varieties of tissue in any section were included in the final Cl estimate, it was found that there was least Cl at the junction of cortex and medulla, that the epithelium of the proximal tubules contained 99–111 mg./100 g. and that the cells of the collecting ducts contained 2,500–2,700 mg./100 g. The deduction made was that the collecting tubules were actively concerned in Cl reabsorption. However, a different interpretation is possible, namely, that a high Cl concentration in the distal and collecting tubules is linked with the final stage of water reabsorption (207). If this is the case, then possibly, the cells of the collecting ducts are one of the sites of action of ADH.

It is evident that, with the information at present available, it is not possible to make any final statement as to the exact point of action of ADH. Fairly certainly the action lies beyond the proximal tubule. It may influence reabsorption in the thin segment; but, more probably, it acts on the cuboidal cells of the distal tubules. Lastly, there is the possibility that ADH may have some effect on the cells of the collecting ducts.

Concentration of posterior lobe extract necessary to produce antidiuresis

In the alligator, Burgess *et al.* (21) found that 5 mU. pitressin/kg. given *intramuscularly* produced a perceptible diminution in the rate of urine flow. This dose, which in the alligator depends for its action on a decreased GFR, may be compared with the effective *intravenous* dose in other animals, in which its action is on the epithelial cells of the tubules. In a dog of 12–15 kg. a marked inhibition of appreciable duration may follow the injection of 1 mU. posterior lobe extract (171). Transient inhibitions follow the intravenous injection of as little as 0.1–0.2 mU. (170, 176, 222). Thus, in the dog full inhibition could be produced by about 0.08 mU./kg. intravenously. In rats 0.4 mU./100g. is effective, and in mice the minimum effective dose is 0.05 mU./20g. (104). In these rodents, then, relatively more extract must be used than in dog or man. Hart and Verney (98) calculated for man that, of hormone released normally from the posterior lobe, the concentration in the plasma which causes partial inhibition of maximum water diuresis is less than one part in 1.66×10^{-10}.

The rate of ADH release from the posterior lobe

Attempts to measure directly the normal output of ADH are hampered by the small amount of hormone that need be present in the blood to inhibit urine flow, and also by the difficulty of obtaining blood direct from the gland. Observations have frequently been made on dehydrated animals in which ADH concentration is likely to be high, and therefore easier to estimate. Often, measurement is made of the quantity of ADH in urine collected over a number of hours. This method has the advantage that samples for assay are easy to collect; also it has been shown that 20–30% of hormone added to the circulating blood was excreted into the urine, where it is fairly stable (122). By such means it has been estimated that dehydrated rats excrete 80 mU. in 24 hr., and that in 6 hr. after NaCl administration rats produce 10–20 mU. of urinary ADH (78). In the urine of severely dehydrated cats 20–400 mU. were found (117). Since none could be found in similar circumstances after hypohysectomy, the pituitary origin of the antidiuretic material was indicated (118). During water diuresis in dogs the amount of ADH liberated by a brief emotional stimulus is about 5–10 mU. (168, 170). Since in dogs 1 mU. is sufficient to cause maximum inhibition of urine flow, it is evident that in the absence of a heavy water load less than this amount in the circulation will be enough to maintain urine flow at the resting rate. Hare, Hickey and Hare (95), using both normal dogs and those with diabetes insipidus, came to the conclusion that usually there was less than 1 mU./100 ml. blood present in the circulation. By giving continuous intravenous infusions of ADH to dogs with diabetes insipidus Shannon (199) found that 1–5 mU./hr. caused the maximal antidiuretic action. Greater amounts than this did not produce a greater antidiuresis. Probably, then, in the normal dog, the release from the gland of 5 mU./hr. would be sufficient to maintain a low rate of urine flow. About the same rate of release seems to be effective in man (139). Normal subjects were kept in a state of full hydration in order to depress endogenous ADH production, and long-continued intravenous infusions were given of varying concentrations of pitressin. For the maximal effect the amount of hormone needed in man was 5 mU./kg./hr.

Measurements have been made of the amount of hormone found in the gland in different animals and man (3, 32, 101–103, 107, 134, 204, 205). These estimates are interesting; for instance, the gland of the newborn human infant contains 20% lessA DH/mg. dry gland than that of the adult. This information, however, tells one nothing of the rate of release of the hormone into the circulation.

The disappearance of ADH from the blood

If, during water diuresis in the dog, 0.5 mU. is injected intravenously its inhibitory effect will have disappeared in 30–40 min. (222). It has also been shown that intravenously injected posterior lobe extract disappears rapidly from the blood and is in part, at least, bound by the plasma proteins and the tissues (106, 122, 221). The bound hormone is, however, antidiuretic on intravenous administration into a hydrated animal, so that the binding does not render it inactive. Possibly, too, there is enzymatic destruction of ADH, especially in the liver (11, 106).

Form and composition of ADH as released from the posterior lobe

As yet it is uncertain in what form ADH is released from the posterior lobe; it may be as a separate hormone, or as part of a large protein molecule whose other portion contains the oxytocic hormone (1, 236). The antidiuretic and oxytocic principles have been prepared separately in highly active form although each of the final fractions was contaminated with the other to the extent of about 1% (123). Against this, there is evidence that by using the gentlest possible methods of chemical concentration and by ultracentrifugation all the active substances concentrate to the same extent (93, 191, 236), suggesting the existence of a single large molecule from which active fractions break off. In man and rat Simon and Nagy (205) and Simon (203) found that the oxytocin and vasopressin content of the glands generally ran parallel. Vaichulis (235) noticed that hormones from autolysed glands showed a greater solubility than those from fresh ones. Both the observations favour the idea of a mother substance. As Stehle (212) points out, even if the gland elaborates a single hormone with multiple activities it does not necessarily mean that that is the form to which the physiological actions are due. If a parent molecule exists it is not known how it is broken into the separate parts. A difficulty in accepting the unitary idea is the report that the various hormones do not occur in the same proportion in all species (74). Recently, too, Cross (41a) has shown in diuretic rabbits that 0.4–1.0 mU. posterior pituitary extract causes an antidiuresis resembling that seen on suckling, whilst the "let-down" of milk, which most workers agree is due to the oxytoxic factor, requires the injections of 50–200 mU. These facts strongly suggest that the two hormones can be independently released from the gland.

It is generally believed that the pressor fraction and ADH are one and the same. Certainly they seem to behave in much the same way towards chemical reagents. Heller (100), however, found that ADH was more stable than the pressor fraction over a wide range of pH, and he prepared solutions with considerable antidiuretic potency, but little pressor action.

The pressor-antidiuretic fraction of posterior lobe extract appears to contain the following amino acids; cystine, tyrosine and perhaps arginine (119, 183, 212, 242).

2. SUBSTANCES WHOSE SITE OF ACTION IS IN THE CENTRAL NERVOUS SYSTEM

Water comes within the definition of a substance found in living tissues, and its concentration in the body is obviously of first importance in regulating the output of ADH. The subject is a vast one and is discussed in a number of books and articles (67, 169, 178, 207, 213). In this place no more than a brief summary of recent information will be given. The quantity of ADH liberated from the posterior lobe depends on the water load of the body (176). The electrolyte concentration of the blood also depends on the water load. Changes in osmolarity of the blood stimulate or inhibit as yet unidentified receptors in the hypothalamus (239, 240). The stimulus, on reaching the supraoptic nuclei, is transmitted down the pituitary stalk by the nerve fibres arising from the cells of these nuclei, and causes the release of more or less ADH by the posterior lobe of the pituitary (58, 67, 178, 179).

However, it is evident that control of ADH production depends also on factors other than water concentration. Urine flow may be reduced at times when it would seem physiological for it to increase or remain high. For instance, an emotional stimulus occurring during the course of a water diuresis results in a diminished rate of urine flow despite the still high water load (168, 170, 171). Certain sensory stimuli also inhibit urine flow during water diuresis (222). Brief exercise, in so far as it has an emotional content, is also inhibitory (193, 239, 240). Finally, the onset of water diuresis or the inhibition of water excretion may be initiated by conditioned reflexes or hypnosis (27, 28, 59, 86, 110, 140, 155, 156). Thus, processes occurring within the central nervous system, either in the cerebral cortex or in lower centres, may influence ADH release. It is, therefore, interesting to examine by what means these internal stimuli are transmitted to the posterior lobe.

Acetylcholine

Two substances are known that are chemical transmitters for at least some peripheral nerve endings, namely, acetylcholine (ACh) and noradrenaline. As regards noradrenaline, there is so far no evidence that it is a central transmitter at synapses on the path to the posterior lobe, though sympathin is found in the hypothalamus in higher concentration than elsewhere in the central nervous system (243). There is growing evidence that ACh is a central transmitter, particularly to the supraoptic nerve cells (58, 66). In 1924 Molitor and Pick (162) drew attention to the fact that choline HCl administered intravenously, delayed the onset of water diuresis. They also remarked that its ester, ACh, was antidiuretic on intravenous, but not on subcutaneous, injection. They ascribed the effect of choline to a change in water distribution. Dikshit (52, 53) found that the injection of ACh into the cerebral ventricle of cats produced effects similar to electrical stimulation of those parts. This latter central action of ACh suggested that perhaps this substance might play a part in the liberation of ADH, and experiments were made on the effect of ACh on urine flow. It was shown that in conscious normal dogs the intravenous injection of ACh during the course of water diuresis resulted in a temporary reduction in the rate of urine flow, but that no inhibition occurred if the posterior lobe of the pituitary had previously been removed (177). An intact pituitary gland, then, was necessary for the antidiuretic action of ACh. In later experiments on dogs under light chloralose anaesthesia, an attempt was made to locate the site of action of ACh by injecting it in a small volume of 0.9 % NaCl solution direct into one or other supraoptic nucleus (179). As a result of this proceeding an established water diuresis could be inhibited, provided the posterior lobe was intact. Injections made into other parts of the hypothalamus were without effect on the rate of urine flow. From these observations it appeared possible that ACh was a transmitter to the supraoptic cells. This explanation became highly probable after prolonged observation of dogs into whose supraoptic nuclei diisopropylfluorophosphonate (DFP) had been injected (58). In these animals after several days of polyuria the daily level of fluid exchange returned to normal, but intravenously administered ACh was without effect on the rate of urine flow for as long as 89–139 days after DFP injection. Towards the end of this number of days there was a period when ACh was sometimes effective and sometimes not. After this length of time ACh resumed its normal inhibiton of the rate of urine flow, as always without alteration in either GFR or RPF (58). In confirmation of the central action of ACh was the observation that if it was injected into the carotid artery rather than into the malleolar vein, far smaller doses were effective in reducing a diuretic rate of urine flow (181). Lastly, nicotine is antidiuretic in normal animals and man, but not in those with diabetes insipidus (22, 25, 33, 144, 219). Thus, there is good reason to believe that ACh is a substance concerned in controlling the output of ADH owing to its transmitter action on the cells of the supraoptic nuclei.

Adrenaline

The part played by adrenaline (or noradrenaline) in the central control of ADH release is complex and its physiological function uncertain. Observations on the emotional inhibition of water diuresis in conscious dogs (171, 239, 240) made it clear that endogenously liberated adrenaline, or adrenaline injected intravenously one half minute before the occurrence of the emotional stimulus, prevented the appearance of the expected antidiuresis. The lack of an antidiuretic response after adrenaline was not due to renal insensitivity to ADH. The action seemed to be a central one and, moreover, independent of changes in systemic blood pressure. Since it has been shown that in the periphery, under certain conditions, ACh and adrenaline are antagonistic (19, 20, 147, 153) there was the possibility that centrally, also, adrenaline interfered with the action of ACh. It was found that if 10–15 μg. adrenaline was injected intravenously just before, or with, a dose of ACh sufficient to decrease a diuretic rate of urine flow, then, on about half of such occasions the antidiuresis failed to appear (57). As ACh alone is rarely ineffective this again suggested a central action of adrenaline, perhaps even on the supraoptic nerve cells. If, instead of giving the drugs intravenously, they were given by intracarotid injection, 2 μg. adrenaline given 11–38 sec. before 0.2 mg. ACh regularly prevented the ADH-releasing action of the latter (Pickford and Watt, unpublished work). Thus, both ACh and adrenaline are concerned with the central control of ADH release. In all probability ACh acts by transmitting impulses to the supraoptic nuclei. The mode of action of adrenaline has yet to be determined. It may directly interfere with the physicochemical reactions of ACh, or there may be local capillary sensitivity to adrenaline, so that the vessels surrounding the supraoptic cells constrict and ACh is prevented from reaching its site of action, or lastly, adrenaline may stimulate other neurones having an influence on the supraoptic cells.

Oxygen lack and carbon dioxide excess

It has been shown that both anoxia and hypercapnia may, by an action on the central nervous system, alter vascular conditions in the kidney and hence the rate of urine flow. In conscious men and animals anoxia generally causes polyuria (202), but in anaesthetized animals oliguria results from severe anoxia, though polyuria may be seen if the anoxia is mild (10, 215). Although it is possible in anaesthetized animals to simulate renal anoxia by the administration of large amounts of adrenaline (230), nevertheless, the adrenal blood vessels can be ligatured and the anoxic response induced as before. The adrenals, therefore, are not necessary for the appearance of the reaction. Intact renal nerves are, however, essential if the antidiuretic effect of anoxia is to be seen. After denervation of the kidneys the rate of urine flow passively follows the systemic blood pressure, but with intact renal nerves anoxia causes a reduction in renal blood flow and the kidney becomes pale and shrunken (230). Possibly there are chemoreceptors in the spinal cord. Franklin, McGee and Ullmann (69) found that the anoxic response of the kidney occurred as usual both after decerebration and, in one instance, after transection of the spinal cord at D4. They did not suggest that the response is always initiated at this level, only that it can occur when these centres alone are intact. There is some evidence that there are cerebral cortical centres which may influence renal blood vessels. Hoff, Kell, Hastings, Gray and Sholes (108) found that prolonged stimulation of the proreus and sigmoideus gyri and orbital surfaces of the cerebral cortex induced a marked renal ischaemia, but they could not suggest under what circumstances these centres would go into action. The last observations were made on cats.

Hypercapnia causes anuria in both dogs and rabbits, even after generous hydration, but if the kidneys are denervated urine is still excreted slowly. In rabbits the surface of the kidney can be seen to pale (55, 69). Draper and Whitehead (55) tried to determine whether the anuria could be accounted for by diversion of the renal blood supply from cortex to medulla. They examined frozen sections of the kidneys of rabbits, into whose blood stream mercuric sulphide had been injected. They found that after denervation the cortical glomeruli of the hypercapnic kidney were clearly visible and must have had a circulation of blood. In the innervated kidney the cortex was pale and, judging by the photograph published, so also were the medulla and the juxtamedullary region. There must, then, have been a closing down of the circulation through the whole kidney. In dogs they were not able to obtain a contrasting picture between innervated and denervated kidney. The analysis of the mode of action of central anoxia and hypercapnia is complicated by the number of centres that may be thrown into activity or inhibited, such as adrenal medullary centres, centres linked with the adrenal cortex or pituitary, and the vasomotor centre as a whole.

3. SUBSTANCES AFFECTING THE CIRCULATION OF BLOOD THROUGH THE KIDNEY

Under this heading are included substances acting directly on the blood vessels, or on the nerve endings supplying the vessels.

Richards and Plant (188) and Dreyer and Verney (56) were amongst the first to point out that intrarenal blood pressure is largely independent of systemic blood pressure. It cannot, therefore, be expected that systemic blood pressure changes, unless considerable, will have much effect on the production of urine. Far more important from this point of view, is what happens in the kidney itself. The production of urine depends in the first place on filtration from the glomeruli into the capsular spaces, and for this purpose the minimum pressure necessary seems to be about 75 mm. Hg (255). Any differential reactivity of the vasa efferentia and afferentia of the glomeruli may cause a change in the rate of urine flow owing to a change in the effective filtration pressure. Further, if the blood supply to some glomeruli is cut off whilst that to others supplying a different or similar type of nephron opens, then again the rate of production and the composition of the final urine may alter.

The substances to be considered in this section are adrenaline, (and noradrenaline), histamine, adenylic acid derivatives, renin and hypertensin, and vasopressin.

Adrenaline and noradrenaline

The injection of adrenaline may lead to either polyuria or oliguria according to the dose and route of administration. In intact man, dog, rabbit, rat and guinea pig the intramuscular or subcutaneous injection of adrenaline results in polyuria. There is also polyuria if the drug is given slowly or in small doses by the intravenous route (41, 73, 111, 145, 229). Thus, polyuria follows if the blood concentration of administered adrenaline is low. With high blood concentrations the rate of urine flow is reduced. The oliguric action is easily seen following a single intravenous injection, when the inhibition is fleeting, or continuous intravenous infusions (57, 177, 193, 222, 223). Unless the kidneys have been denervated there is no correlation between systemic blood pressure and urine flow. Therefore, if the rate of urine flow is seen to change, it is probably because the adrenaline has had an intrarenal effect. There is no evidence that it has any specific action on the cells of the tubules. Richards and Plant (188), observing that in the perfused rabbit kidney adrenaline increased both the resistance to perfusion and the volume of the kidney, suggested that the polyuric effect was due to increased filtration brought about by constriction of the efferent arterioles of the glomeruli, accompanied by dilatation, or no change, in the afferent arterioles. Winton's (254) observations on the isolated kidney of the dog seem to bear out this suggestion. He found that low concentrations of adrenaline caused an increase in the rate of urine flow accompanied by only a slight reduction in the blood flow, and an increase in filtration pressure. Greater concentrations of adrenaline decreased the rate of urine flow with a fairly marked reduction in blood flow and glomerular pressure. The reduction in renal blood flow has also been observed in intact animals by the use of the thermostromuhr (99, 209, 234). Smith (207), citing unpublished work of Gomez, thinks that adrenaline diuresis cannot be explained by a preferential efferent vasoconstriction. On the basis of clearance observations made in man he believes that adrenaline constricts the venules, and so prevents water reabsorption. Also the venular constriction, by altering the interstitial pressure and water content, would explain the increase in volume noticed by Richards and Plant. Adrenaline antidiuresis is easily explained by a general vasoconstriction of the smaller renal arterioles, and venules according to Smith (35, 89, 229, 254).

Clearance measurements show that with doses of adrenaline sufficient to produce oliguria (about 2μg./min.) both GFR and RPF are reduced in dogs (182). Some workers believe that denervation of the kidneys sensitises them to the action of large doses of adrenaline (133, 152). Others have not observed this phenomenon (182).

Eränkö and Karvonen (60) find that if large (0.5 mg.) doses of adrenaline are given intravenously to conscious dogs a biphasic antidiuretic effect is seen, the second part of which is long-lasting and takes 20 min. to reach its maximum. Urine collected during the antidiuretic phases contains an antidiuretic substance, as judged by its effect when injected intravenously into the dog from which the urine was collected. The authors think that large doses of adrenaline cause the release of ADH from the posterior lobe. The work is still in its early stages. Possibly, the renal ischaemia that must follow the use of such large doses of adrenaline is the cause of the prolonged antidiuresis. Verney and Vogt (241) noticed that prolonged antidiuresis could result from a 5–120 sec. compression of the renal artery in the conscious dog, and that the antidiuresis was associated with hypertension in unilaterally nephrectomized animals.

From the information available it seems that noradrenaline acts on the kidney more powerfully than adrenaline, but in the same way, at any rate as far as the excretion of water is concerned (24, 182). In rats noradrenaline increases absolutely the excretion of Cl, a phenomenon not seen after the administration of adrenaline (61, 111).

Histamine

A consideration of the action of histamine on the kidney is important since it has repeatedly been shown that histamine is released into the circulation from all injured tissues, whether the injury is mechanical or due to allergy, and that the liberated histamine can cause profound disturbances at many sites.

Most observations on the kidneys have been made after subcutaneous administration of histamine, and the results vary. In man, any antidiuretic effect of 0.15–0.7 mg. is generally slight or absent. The subjects examined were either normal, hypertensive or suffering from diabetes insipidus (75, 187, 250). In dogs Molitor and Pick (162) found that antidiuresis followed a total dose of 1 mg., whereas Theobald and White (223) observed no renal action after a total dose of 0.5 mg. Gilman and Kidd (79) gave 2.5 μg./kg. intravenously to dogs and did observe antidiuresis. The writer has noticed slight or sometimes marked antidiuresis follow the intravenous injection of 20 μg. into a dog of 20 kg. weight. The same variability in response has been noticed in rabbits (104, 162). Most workers agree that the changes in renal function are not directly dependent on the fall in systemic blood pressure that occurs. Reubi and Futcher (187) measured the PAH and mannitol clearances in man after subcutaneous histamine and found that in normal subjects both GFR and RPF fell. This, they believe, was due to afferent arteriolar constriction. The renal volume changes seen by Dale and Laidlaw (44) could also be interpreted as due to arteriolar constriction. Bjering (14) found in man that creatinine and urea clearances fell, and thought that both glomerular and tubular vessels constricted. It is evident that the exact mode of action of histamine is not settled. In man histamine induces general arteriolar constriction, unlike adrenaline the response to which shows regional differences. This fact, with the evidence mentioned, makes it reasonable to suppose that histamine induces arteriolar constriction in the kidney. The injection of histamine also causes the release of adrenaline from the adrenal medulla and this may influence the final result, either by adding to the constrictor effect or, in smaller amounts, by partially counteracting the histamine antidiuresis.

There are some data on the effects of certain types of shock on the kidneys of dogs. After acute haemorrhagic and traumatic shock PAH clearances are normal until the RPF falls very low (37, 174). At first sight this might suggest that histamine is not a factor in causing the oliguria of shock, but these observations were acute ones made on anaesthetized dogs, whereas in man death tends to occur a few days after injury. Chronic experiments show that the kidney of the dog is capable of recovery if its circulation has not been cut off for too long, or too frequently in a short space of time (37, 174). Dog's kidneys seem to be more resistant than those of man, in whom a relatively short-lived ischaemia may be so damaging to the kidney that its functional recovery is too slow to permit survival of the rest of the organism. Cort and Barron (38) found that in cats the anuria of trauma could be relieved by the injection of local anaesthetics into the splanchnic nerve or the spinal roots supplying the kidney. It is possible, then, that ischaemia and anuria following injury may be partly reflex in origin and partly due to excessive amounts of histamine and other vasoactive substances present in the blood.

Adenylic acid derivatives

These are substances which, like histamine, are known to be released into the circulation from injured tissues. Tested on normal men adenosinetriphosphate reduces the volume output of the kidney (87). However, all these substances are powerful vasodilators for the kidney (124) and their antidiuretic action has been found to run parallel with their effect on the general blood pressure and to be rapidly reversible (113). Any antidiuresis occurring as a result of the presence of these substances is probably due to the general vasodilatation and the fall in blood pressure that they cause.

Renin and hypertensin

In conscious hydrated rabbits renin first causes a brief antidiuresis which is followed by diuresis (115). Pickering and Prinzmetal (175) attribute the antidiuretic effect to an action on the glomerular vessels and a reduction in the rate of filtration. Results on dogs vary to some extent in the hands of different workers. Pickering and Prinzmetal observed a well-maintained antidiuresis in the conscious dog, whilst Merrill, Williams and Harrison (160) generally found a slight or well-marked diuresis as the result of the intravenous administration of renin, whether or not the dogs had been hydrated. The latter workers noticed that the kidney volume increased at the same time that the renal blood flow decreased, an effect similar to that seen after adrenaline (188).

In both dog and rabbit hypertensin has the same effect on the kidneys as renin. This similarity of action is not surprising since in all probability renin is merely the enzyme which, with the hypertensinogen of the plasma, forms hypertensin (17, 172). In the main, the action of these two substances appear to be diuretic rather than antidiuretic. What function, if any, they have in the normal organism is not known.

Vasopressin, other vasoactive substances, and the intrarenal redistribution of blood

Since it is probably impossible to separate the ADH of the posterior pituitary from the pressor fraction, and since the former is effective in far smaller doses than the latter, the examination of the unmixed action of vasopressin on the kidney is not possible. Given in doses at least 7 times larger than those necessary to produce antidiuresis, vasopressin decreases the renal blood flow in dogs and frogs (244). In mammals generally the vasopressor action seems to be of pharmacological rather than physiological interest to the kidney.

The suggestion has been made by Trueta, Barclay, Daniel, Franklin and Prichard (231) that posterior pituitary substances and adrenaline alter renal function by causing a redistribution of blood in the kidneys, and that the antidiuretic effect of the former is due to constriction of cortical arterioles with a transference of blood to medullary nephrons, where the long thin segment reabsorbs a large part of the tubular fluid. A somewhat similar suggestion was originally made by Frey (70, 71). Certainly the juxtamedullary nephrons are arranged differently from the cortical. As Huber (114) showed, the former have a much longer thin segment and the distal tubule (in rabbits) is about half as long as that in the cortical nephrons. Also the pattern of the surrounding capillaries is different, and the efferent arteriole of the juxtamedullary glomerulus is larger than the afferent (231). The work of Trueta *et al.* (231) was done mainly on rabbits, rats and cats, and they observed the renal circulation radiologically using thorotrast as the contrast medium. Recently, Daniel, Peabody and Prichard (45) have extended the observations to include dogs, sheep and monkeys. In some, but not all, individuals of all these species, under the conditions of their experiments, a closing down of the cortical blood supply was observed. The effective conditions were direct or reflex stimulation of the renal nerves, tissue trauma by the application of a tourniquet to one or both hind limbs, intravenous pituitrin 0.1–0.17 units/kg. rabbit, intravenous pitressin 0.2–20 units/kg. rabbit, intravenous adrenaline 0.1–0.17 mg./kg. rabbit, and equivalent doses for other animals. Daniel *et al.* also gave 5 μg. adrenaline into the aorta. As far as antidiuresis is concerned the doses used were monumental and might well cause a blood diversion which would not be seen with smaller, but effectively antidiuretic, doses. Given intravenously 5 μg. adrenaline is enough to cause maximal antidiuresis. Given intra-aortically, as was done by Daniel *et al.*, this dose would mean a very high concentration in the renal blood. Having seen that large doses had a renal vascular action, it is a pity that Trueta *et al.* made no attempt to test the response to physiological amounts of the extracts. There is a further criticism of their suggestion that hormone antidiuresis is vascular and not specifically tubular in origin, namely, that after denervation of the kidneys they found no change in blood distribution in the kidneys. It has frequently been shown that ADH is an antidiuretic in the denervated as in the normal kidney (170, 171, 194, 223) Thus, it is unlikely that a hormonal mechanism causes the vascular changes, or that the vascular changes have anything to do with antidiuresis. Other workers using both similar methods to and different methods from those of Trueta *et al.* have not always come to the same conclusions. It is easy to cause ischaemia of the whole kidney, less easy to cause cortical ischaemia only. However, the latter has been induced fairly often in rabbits, more rarely in rats and dogs, and so far, not at all in healthy men, or in men suffering from essential hypertension or congestive cardiac failure (6, 83, 112, 157, 216). In man it is obviously impossible to use enormous doses of pitressin, and information regarding renal blood flow and function has to be obtained indirectly by means of clearance measurements. There is, in fact, no evidence that *normal* renal excretory function in man, dog or a number of other mammals in any way depends on intrarenal redistribution of blood.

This is not the place to discuss general renal physiology, but the question of blood redistribution in the organ cannot be left here. Three facts, amongst others, have to be taken into consideration; there is no reason to invoke, nor evidence for, intrarenal blood redistribution as a cause of pituitary or adrenaline antidiuresis when small but adequate doses are used; the kidney is richly supplied with nerves, but it has yet to be proved that these are concerned with renal excretory activity; under certain experimental and clinical conditions the blood supply to the cortical nephrons is diminished. On the last point the work of Trueta *et al.* has already been mentioned. Goodwin, Sloan and Scott (83) could at times, and particularly by direct stimulation of the renal nerves, induce cortical ischaemia in the kidneys of rabbits, cats, dogs and monkeys. Hoff, Kell, Hastings, Gray and Sholes (108) and Hoff, Kell, Hastings, Sholes and Gray (109) found that prolonged intermittent stimulation of certain gyri of the cerebral cortex resulted in renal cortical, but not juxtamedullary, ischaemia. To the writer there are only two possible ways of accounting for the conflicting evidence as to the importance of intrarenal blood redistribution for renal function. Either cortical ischaemia with medullary engorgement is a wholly abnormal phenomenon, occurring only in response to extreme provocation, or it is normal and connected with an endocrine activity of the kidney, not with its excretory function. In favour of the first possibility is the fact that extreme methods have to be applied to cause renal cortical ischaemia. In favour of the second are the following points. We know that a hypertensive substance, renin, is found in the kidney, and that a vasoexcitor material (VEM) (201) can be collected from it. We know, too, that in some ill-understood way, there is a connexion between the kidney and certain hypertensive states, both clinical and experimental. Renal nerve supply and occasional intrarenal blood redistribution may be concerned with the production of these materials. Under what circumstances this system is activated, why it is more reactive in some species and some individuals than in others, and what place it holds in the normal, as against the pathological, economy are quite unknown.

Recently, a highly vasoactive and antidiuretic substance has been extracted from the posterior salivary glands of certain octopi, and from some viscera of mammals, and named enteramine (60b). Its identity with 5-hydroxy-tryptamine has been established (60a). By means of thiosulphate and *p*-aminohippuric acid clearance measurements on rats and dogs it was found that its antidiuretic action was due to powerful constriction of the glomerular afferent arterioles, and that it was effective in doses equivalent to 2 mg. fresh salivary tissue per 100 g. rat. The minimal effective dose for antidiuresis in dogs was not determined (60c, 60d).

4. MISCELLANEOUS ANTIDIURETIC SUBSTANCES AND CAUSES OF ANTIDIURESIS

Ferritin

It has for a long time been recognised that the liver stands in a special relationship with the kidney (76). Hepatic disease is accompanied by disturbance in water balance and either oliguria or, as in dogs with an Eck fistula (40), polyuria may result. The liver, too, seems to be involved in the first stages of water distribution after water ingestion (2). Finally, a large number of workers have extracted antidiuretic substances from the liver (9, 82, 137, 159, 195, 224, 245). One of these antidiuretic substances, originally called VDM (vasodepressor material), has now been identified as ferritin. It can be formed in the spleen and muscles as well as in the liver, and its production seems to depend on hypoxia of the organs concerned. Ferritin is found in the circulation in renal hypertension in dogs, and in man in essential hypertension, congestive heart failure and the hyporeactive phase of traumatic or haemorrhagic shock, but not in healthy men (201). The activity of ferritin runs parallel with its nitrogen content, that is, with its protein and not its iron-containing moiety (158). The suggestion is that ferritin appears in the blood in shock as the result of hepatic ischaemia (201). The normal liver can inactivate ferritin, but an anoxic liver loses this power, so that increasing amounts accumulate in the blood. The antidiuretic action of ferritin occurs without alteration in blood pressure, GFR or RPF, and is not seen in dogs with diabetes insipidus (207). Presumably, therefore, its action is mediated through the posterior lobe of the pituitary. It is unknown what function ferritin has in the normal animal.

Hepato-renal syndrome

In contrast to the polyuria that occurs in some hepatic diseases, in others oliguria and ascites predominate, but there is no evidence that the water retention is due to the production of ferritin. In hepatic cirrhosis the presence of low plasma albumin and ascites do not run parallel. In some cases treatment with liver extract lessened the ascites without raising the plasma albumin, and albumin could be administered without benefit to the oliguria (185, 249). Thus, water retention cannot be due simply to the osmotic effect of low plasma protein. Ralli (185) and her colleagues found that urine from patients with hepatic cirrhosis often had an abnormally high content of antidiuretic substance as judged by the water excretion rate of rats after subcutaneous administration of the urine. This antidiuretic substance appeared to have some of the characteristics of commercial pitressin (217). A similar substance could also be found in the urine in cases of infective hepatitis, and diminished in amount with the approach of convalescence (136). Ralli suggested that water was retained in these cases because liver damage had reduced the power of that organ to inactivate ADH. In favour of this idea was the finding that ADH was effectively inactivated by an enzyme that could be extracted in more abundance from liver than from spleen, whole blood or muscle (11, 62). Also, in rats whose livers were damaged by a high fat-low protein diet the power of excreting water was markedly depressed and delayed, and moreover, could be improved by supplementing the diet with liver extract and NaCl (142). These animals, like patients with cirrhosis, excreted large amounts of antidiuretic substance in the urine. Gopalan (85) states that he found antidiuretic material in the urine of men suffering from nutritional oedema. The above results could all be interpreted as supporting Ralli's suggestion that excess ADH is the cause of hepatic ascites and oedema. However, she and her co-workers themselves found certain facts that do not agree with this hypothesis. First, they found that in hepatic cirrhosis all renal functions are depressed (141), a phenomenon which is only seen if very large amounts of ADH are administered, and secondly and more important, they are quoted by Smith (207) as stating that the antidiuretic substance obtained from patients with cirrhosis will not inhibit water diuresis in the dog when given intravenously, though it is antidiuretic on intraperitoneal administration to the rat. This at once suggests that the problem should be reinvestigated using the rat intravenous method, and bearing in mind the errors that may arise when other routes of administration are used (49). In other words, it is an interesting suggestion that water retention in hepatic disease is due to inadequate ADH destruction, but it is very far from proven.

The abnormal water metabolism in liver disease can more simply be explained as due to altered electrolyte balance. The retention of water and the ascites are not necessarily due to renal disease, since with improvement in the hepatic condition water excretion may return to normal (141). Farnsworth (63) believes that in congestive heart failure and hepatic cirrhosis some disturbance in Na and Cl excretion precedes oedema formation, and not the reverse. Goodyer, Relman, Lawrason and Epstein (84) came to the same conclusion as the result of observations on the rate at which infused NaCl was excreted by normal subjects, and by patients with and without ascites. They believe that there is evidence for specific impairment of the renal mechanism for the excretion of Na in patients with cirrhosis accompanied by ascites and oedema. They made no suggestion as to the cause of the increased Na reabsorption.

Adrenal cortical disorders

In Addison's disease ingested water is only slowly excreted and there is a tendency for water intoxication to appear. Birnie, Jenkins, Eversole and Gaunt (13) suggested that the liver may, in part, be the cause of the oliguria. At first they thought that there was a sensitisation to ADH. Later Birnie (11) found that rat liver extracts made after adrenalectomy of the donors were less able to inactivate ADH than extracts of normal livers, so that the apparent sensitisation was, perhaps, due to a diminished destruction of ADH. It was also found that after adrenalectomy the antidiuretic titre of the serum was raised, but could be reduced again by the administration of adrenal cortical extract, deoxycorticosterone or NaCl (12). The tests were made on rats and the serum injected intraperitoneally. It was thought that the increased antidiuretic activity of the serum was due to the already mentioned deficiency in the system which usually destroys ADH. However, it should be noted that although treatment reduced the antidiuretic titre of the serum, water excretion did not return to normal. Possibly therefore, there was at no time a real change in the ADH content of the serum, but only of some substance which had been acting as an augmentor to the endogenously formed hormone.

The changes of water excretion in Addison's disease are most readily explained as due to the inevitable marked electrolyte disturbance and low blood pressure. The subject of the adrenal cortex and water metabolism is too vast to be further considered here, and references may be found in Gaunt, Birnie and Eversole (72a) and in Smith (207).

Antidiuretic substances found in pregnancy

For the greater part of normal pregnancy there seems to be neither specific water retention nor measurable amounts of antidiuretic substances in serum or urine (50, 54, 131, 151, 251), but late in apparently normal pregnancies some women show a lessened capacity for water excretion, and the same thing has been observed during parturition (50, 151). Some observers claim that pregnant serum contains a substance antagonistic to ADH and melanophore hormone, and particularly is this antihormone found in the first part of normal pregnancy. Very little of this substance can be found in the serum of women who are not pregnant (135, 198).

There is a considerable divergence of opinion as to what occurs in eclampsia and pre-eclampsia. Anselmino, Hoffman and Kennedy (5) thought that they had demonstrated the presence of an antidiuretic substance in the blood of eclamptic women, and that this substance was of posterior pituitary origin. Theobald (222) showed that their methods were not reliable, and that their claims could not be substantiated, particularly with regard to the origin of the antidiuretic material. A number of other workers agreed with Theobald (30, 116, 131, 143). On the other hand, Teel and Ried (220) found that there was an antidiuretic substance in the urine of eclamptic women, especially during the phase of acute water retention. The quantity of antidiuretic material found was correlated with the degree of water retention and not with the hypertension or albuminuria. Others (91, 92, 195) too, have found an antidiuretic substance in the urine in pre-eclampsia, but think that it differs from true ADH. Still others believe that they have shown the existence of an increased sensitivity to vasopressin in pre-eclampsia (29, 50) and that in this condition patients react to injections of vasopressin by a rise in blood pressure and fall in urine volume which is far greater than that seen in normal women. Kustner (135) believes that the antihormone present in normal pregnancy is lacking in eclampsia.

In the general confusion it seems clear that excessive production of ADH with primary water retention is not the *cause* of eclampsia or hypertension in pregnancy. It remains to be decided whether antidiuretic substances of unknown origin are formed in considerable amounts in eclampsia and pre-eclampsia, or whether their apparent discovery is due to the use of unsuitable test methods. The latter seems the more probable.

Various steroids

There is general appreciation of the fact that in certain phases of the sexual cycle of females some alteration or disturbance of water metabolism often occurs. In some species an obvious alteration is physiological and is accompanied by water retention (132); in others water retention is not apparent, and, if it does occur, may be abnormal. The question arises whether the known changes in steroid balance are directly or indirectly the cause of altered water metabolism.

Zuckerman, Palmer and Hanson (260) made observations on the water content of some of the tissues of rats after the administration of a single dose of oestrogens. The measurements were made at 6-hourly intervals for 3 days. A biphasic change in water content was found, with an inverse relationship between uterus, vagina and skin and other tissues. The uterus returned to its normal weight in 72 hr. and all other organs in 48 hr. As no figures were given for the total water content of the animals or their body weight changes, it is not possible to say whether there was an actual water retention or only a redistribution of water in the body. Guthkelch and Zuckerman (90) compared the red blood cell counts during phases of the sexual cycle in pig-tailed macaques and rhesus macaques. The pig-tailed species displays sexual skin swelling, and at such a time the red blood cell count increased from 4.5 to 5.75 million with subsidence to normal at the next mid-cycle. There was also a diminished urine output during the phase of sexual skin swelling. The rhesus displays colour changes but no skin swelling; nevertheless, a similar concentration of red blood cells occurred. There was no clear evidence of body weight change and true water retention. When the rhesus was ovariectomised and given oestrone a rise in the red blood cell count was seen during the early follicular phase. No generalisation was possible on the basis of these results. Witten and Bradbury (258) made blood counts and blood volume estimates on women during the intermenstrual period in order to avoid the complication of blood loss. After injections of oestrone or oestradiol propionate they found that the red blood cell count fell to an extent that could be accounted for by the simultaneous increase in blood volume. Friedlander, Laskey and Silbert (72) state that, in women, ovariectomy results in a marked decrease in blood volume, and that administration of oestrogenic preparations increases it again. These results are in contrast to those of Zuckerman, but this may be due to species differences. The difference between pig-tailed and rhesus macaque has already been mentioned, and Friedlander, Laskey and Silbert themselves noted that in humans when the blood volume rose the plasma volume sometimes fell, whereas in cats both blood and plasma volume rose.

In observations made on dogs, Thorn (226, 227, 228) and his colleagues found that whether or not the dogs were adrenalectomised the administration of oestrone, progesterone, pregnanediol or testosterone led to the retention of Cl, Na and water. The same workers made balance studies on women and found that Cl, Na and water were retained at two periods of the menstrual cycle, during the premenstrual phase and in mid-cycle (228). With the onset of menstruation the retained water and electrolytes were excreted. Similar changes were seen in women after hysterectomy, but not after both hysterectomy and ovariectomy. The changes in water and electrolyte metabolism were, then, probably related to the ovary and oestrogens. There were, however, indications that the weight gain depended on a cyclical increase in hunger and thirst, since by controlling the diet the weight gain could be avoided. Some women show a well-recognised state of premenstrual tension. This is related to an increase in weight with water retention and, sometimes, obvious general oedema. The basal metabolic rate may be low. The fall in BMR, but not the oedema, can be prevented by thyroid administration (8). It has also been noticed that the administration of progesterone can give rise to all the signs of premenstrual tension in those liable to the condition (77). Progesterone has also been shown to prevent the usual post-partum loss of water in mice (46, 47). Frank (68) states that irradiation of the ovaries led to a 3-year cure in patients suffering from premenstrual tension. These observations all point to changes in water metabolism being dependent on the balance of steroids in the body, but do not show how it is brought about. Thomas (225) thought that the tissues were made hydrophilic by a rise in oestrogen concentration, but suggested no cause for the hydrophilia. A number of workers believe that Na retention is the first event, and water retention is secondary (34, 88, 189, 218). From the observations of Thorn and Engels (226) that changes in Na and Cl excretion due to administration of sex hormones were seen in both normal and adrenalectomised dogs, it appears that adrenal hormones are not necessary for the changes that occur. In all the conditions mentioned water retention is best explained as due to a steroid induced alteration in electrolyte balance.

Two other possibilities may be briefly considered, first, the action of oestrogens on the anterior lobe of the pituitary, and second, their action on the posterior lobe. Oestrogens are known to depress certain activities of the anterior lobe, and so may result in depression of renal function (180, 253). Shapiro (200) found that following large daily injections of oestradiol benzoate to 5 patients there was marked decrease in their urinary output, and that the same reduction was seen in a patient with diabetes insipidus. He suggested that anterior lobe activity had been sufficiently depressed to disturb renal function.

Cavallero, Corbetta and Malandra (31) found that in ovariectomised rats there was a reduction in the ADH content of the pituitary that was not seen if the operated animals had been treated with oestrogens. They suggested that with a changed steroid balance more ADH was produced. Martin, Herrlich and Fazekas (154) postulate that, following adrenalectomy, an initial stimulation of the posterior lobe is due to early dehydration, with a secondary augmentation due to electrolyte imbalance.

5. SUMMARY

Urine output may be reduced and water retained in the body for a number of reasons, many of which affect water only secondarily. For instance, any process or substance which alters the electrolyte balance will almost certainly also affect water excretion. As examples may be mentioned hepatic and adrenal disorders, and the changes in steroid balance that occur during the sexual cycle. More directly, water excretion may be diminished when the blood pressure in a part or the whole of the kidney is reduced, or where the renal vessels undergo general constriction. Such renal vascular changes may be caused by substances in the circulating blood, or by activity of the renal nerves. Finally, water excretion may be reduced by the specific action of the antidiuretic hormone of the posterior lobe of the pituitary, and also, therefore, by all substances and conditions that increase the rate of release of the hormone into the circulation. A study of the literature makes it evident that ADH is the only known tissue substance whose normal function it is to control water excretion *per se*, and that it does so by an action on the cells of the renal tubules.

REFERENCES

1. Abel, J. A., Rouiller, C. A. and Geiling, E. M. K. Further investigations on the oxytocic-pressor-diuretic principle of the infundibular portion of the pituitary gland. J. Pharmacol. & Exper. Therap., **22**: 289-316, 1923.
2. Adolph, E. F., Gerbasi, M. J. and Lepore, M. J. Redistribution of water following transfusions and infusions. Am. J. Physiol., **107**: 647-666, 1934.
3. Ames, R. G. and Van Dyke, H. B. Antidiuretic hormone in the urine and pituitary of the kangaroo rat. Proc. Soc. Exper. Biol. & Med., **75**: 417-420, 1950.
4. Ames, R. G. and Van Dyke, H. B. Antidiuretic hormone in the serum or plasma of rats. Endocrinology, 1952 Endocrinology, **50**: 350-360, 1952.
5 Anselmino, K. J., Hoffmann, F. and Kennedy, W. P. The relation of hyperfunction of the posterior lobe of the hypophysis to eclampsia and nephropathy of pregnancy. Edinburgh M. J., **39**: 376-388, 1939.
6. Arcadi, J. A. and Farman, F. Experimental studies and clinical aspects of the renal circulation. J. Urol., **62**: 756-761, 1949.
7. Arnold, O. Über blutdrucksteigernde und diuresehemmende Substanzen im Harn. Arch. f. exper. Path. u. Pharmakol., **190**: 360-371, 1938.
8. Atkinson, A. J. and Ivy, A. C. Menstrual oedema. J. Am. M. A., **106**: 515-517, 1936.
9. Baez, S., Mazur, A. and Shorr, E. The antidiuretic action of crystalline VDM (ferritin, apoferritin). Federation Proc., **8**: 7, 1949, and Am. J. Physiol., **162**: 198-212, 1949.
10. Berger, E. Y., Galdston, M. and Horwitz, S. A. The effect of anoxic anoxia on the human kidney. J. Clin. Investigation, **28**: 648-652, 1949.
11. Birnie, J. H. The inactivation of posterior pituitary antidiuretic hormone by liver extracts. Federation Proc., **9**: 12, 1950.
12. Birnie, J. H., Eversole, W. J., Boss, W. R., Osborn, C. M. and Gaunt, R. An antidiuretic substance in the blood of normal and adrenalectomised rats. Endocrinology, **47**: 1-12, 1950.
13. Birnie, J. H., Jenkins, R., Eversole, W. J. and Gaunt, R. An antidiuretic substance in the blood of normal and adrenalectomised rats. Proc. Soc. Exper. Biol. & Med., **70**: 83-85, 1949.
14. Bjering, T. The influence of histamine on renal function. Acta med. Scandinav., **91**: 267-278, 1937.
15. Blumgart, H. L. The antidiuretic effect of pituitary extract applied intranasally in a case of diabetes insipidus. Arch. Int. Med., **29**: 508-514, 1922.
16. Bohn, H. and Coester, C. Untersuchungen des Blassen Hochdrucks. Ztschr. f. klin. Med., **126**: 593-599, 1934.
17. Braun-Menendez, E., Fasciolo, J. C., Leloir, L. F., Munoz, J. M. and Taquini, A. C. Renal Hypertension. C. C Thomas, Springfield, Illinois, 1946.
18. Brun, C., Knudsen, E. O. E. and Raaschou, F. On the cause of post-syncopal oliguria. Acta med. Scandinav., **132**: 486-500, 1945.
19. Bulbring, E. The action of adrenaline on transmission in the superior cervical ganglion. J. Physiol., **103**: 55-67, 1944.
20. Bulbring, E. and Burn, J. H. Vascular changes affecting the transmission of nervous impulses. J. Physiol., **97**: 250-264, 1939.
21. Burgess, W. W., Harvey, A. M. and Marshall, E. K. The site of the antidiuretic action of pituitary extract. J. Pharmacol. & Exper. Therap., **49**: 237-249, 1933.
22. Burn, G. P. and Grewal, R. S. The antidiuretic response to, and excretion of, pituitary (posterior lobe) extract in man, with reference to the action of nicotine. Brit. J. Pharmacol., **6**: 471-482, 1951.
23. Burn, J. H. Estimation of the anti-diuretic potency of pituitary (posterior lobe) extract. Quart. J. Pharm. & Pharmacol., **4**: 517-529, 1931.
24. Burn, J. H. and Hutcheon, D. E. The action of noradrenaline. Brit. J. Pharmacol., **4**: 373-380, 1949.
25. Burn, J. H., Truelove, L. H. and Burn, I. The antidiuretic action of nicotine and of smoking. Brit. M. J., **1**: 403-405, 1945.
26. Buschke, F. Experimentalle Beitrage zum Wirkungs mechanismus des Hypophysins auf den Wasser- und Chloridwechsel. Arch. f. exper. Path. u. Pharmakol., **136**: 43-51, 1928.

27. Bykow, K. M. and Alexejew-Berkmann, I. A. Die Ausbildung bedingte Reflexe auf Harnausschiedung. Arch. ges. Physiol., **224**: 710–721, 1930.

28. Bykow, K. M. and Alexejew-Berkmann, I. A. Die Ausbildung bedingte Reflexe auf Harnausschiedung. Arch. ges. Physiol., **227**: 301–308, 1931.

29. Byrom, F. B. Effect of oestrogenic and other sex hormones on the response of the rat to vasopressin. Lancet, **1**: 129–131, 1938.

30. Byrom, F. B. and Wilson, C. Alleged pituitary origin of eclamptic and pre-eclamptic toxemias of pregnancy. Quart. J. Med., **27**: 361–368, 1934.

31. Cavallero, C., Corbetta, S. and Malandra, B. The antidiuretic power of rat neurohypophysis after ovariectomy and replacement therapy. Arch. internat de pharmacodyn. et de thérap., **87**: 366–370, 1951.

32. Cavallero, C. and Zanchi, M. The antidiuretic hormone of the posterior lobe of the human hypophysis. J. Path. & Bact., **65**: 249–252, 1951.

33. Chalmers, T. M. and Lewis, A. A. G. Stimulation of the supraoptico-hypophyseal system in man. Clin. Sc., **10**: 127–135, 1951.

34. Chart, J. J., Shipley, E. G. and Gorden, E. S. Evidence for a sodium retaining factor in toxemia of pregnancy. Proc. Soc. Exper. Biol. & Med., **78**: 244–246, 1951.

35. Chasis, H., Ranges, H. A., Goldring, W. and Smith, H. W. Control of renal blood flow and glomerular filtration in normal man. J. Clin. Investigation, **17**: 683–697, 1938.

36. Coester, C. Über biologische wirksame Substanzen im Harn. II und III Mitteillungen. Ztschr. f. klin. Med., **128**: 656–664, 1935.

37. Corcoran, A. C. and Page, I. H. Effects of hypotension, hemorrhage and of blood transfusion on renal function in dogs. J. Exper. Med. **78**: 205–224, 1943.

38. Cort, J. H. and Barron, D. H. Neural control of renal shunt. Federation Proc., **7**: 23, 1948.

39. Craig, F. N. The action of pituitary extract on urinary secretion. Quart. J. Exper. Physiol., **15**: 119–154, 1925.

40. Crandall, L. A. and Roberts, G. M. Increased water exchange following Eck fistula in dogs. Am. J. Physiol., **117**: 318–327, 1936.

41. Credner, K. Über die Beeinflussung der Diurese durch Ephedrin, Sympatol, Veritol und Pervitin. Arch. f. exper. Path. u. Pharmakol., **206**: 188–190, 1949.

41a. Cross, B. A. Suckling antidiuresis in rabbits. J. Physiol., **114**: 447–453, 1951.

42. Cutting, M. and McCance, R. A. The metabolism of kidney slices from new-born and full-grown rats. J. Physiol., **104**: 288–298, 1946.

43. Cutting, M. and McCance, R. A. The metabolism of kidney slices from new-born and mature animals. J. Physiol., **105**: 205–214, 1946.

44. Dale, H. H. and Laidlaw, P. P. The physiological actions of B-iminazolylethylamine. J. Physiol., **41**: 318–344, 1910.

45. Daniel, P. M., Peabody, C. N. and Prichard, M. M. L. Cortical ischaemia of the kidney with maintained blood flow through the medulla. Quart. J. Exper. Physiol., **37**: 11–18, 1952.

46. Dewar, A. D. Possible role of progesterone in the water retention of pregnancy in the mouse. J. Physiol., **112**: 53P, 1950.

47. Dewar, A. D. and Newton, W. H. The nature of the weight loss following parturition and "pseudo-parturition" in the mouse. J. Physiol., **111**: 36–37P, 1950.

48. Dicker, E. A propos de l'hypertension artèrielle consécutive à une entrave de la circulation rénale. Compt. rend. Acad. Sc., **125**: 1046–1047, 1937.

49. Dicker, S. E. and Tyler, C. Antidiuretic titre of plasma from the internal jugular vein of children. J. Physiol., Proceedings: J. Physiol., **117**: 28–29 P. 1952.

50. Dieckmann, W. J. and Michel, H. L. Vascular renal effects of posterior pituitary extract in pregnant women. Am. J. Obst. & Gynec., **33**: 131–137, 1937.

51. Dietel, F. G. Hypophysenhinterlappen-sekretbindene Stoffe im Schwangerserum. Klin. Wchnschr., **12**: 1683–1686, 1933.

52. Dikshit, B. B The production of cardiac irregularities by excitation of the hypothalamic centres. J. Physiol., **81**: 382–394, 1934.

53. Dikshit, B. B. Action of acetylcholine on the "sleep centre". J. Physiol., **83**: 42P, 1935.

54. Dill, L. V., Isenhour, C. E., Cadden, J. F. and Robinson, C. E. Glomerular filtration and renal blood flow in normal patients following toxemias of pregnancy. Am. J. Obst. & Gynec., **44**: 66–72, 1942.

55. Draper, W. B. and Whitehead, R. W. The phenomenon of diffusion respiration. Anesth. & Analg., **28**: 307–318, 1949.

56. Dreyer, N. B. and Verney, E. B. The relative importance of the factors concerned in the formation of urine. J. Physiol., **57**: 451–456, 1923.

57. Duke, H. N. and Pickford, M. Observations on the action of acetylcholine and adrenaline on the hypothalamus J. Physiol., **114**: 325–332, 1951.

58. Duke, H. N., Pickford, M. and Watt, J. A. The immediate and delayed effects of diisopropylfluorphosphonate injected into the supraoptic nuclei of dogs. J. Physiol., **111**: 81–88, 1950.

59. Eagle, E. Conditioned inhibition of water diuresis. Amer. J. Physiol., **103**: 362–366, 1933.

60. Eränko, O. and Karvonen, M. J. Adrenaline antidiuresis in water-loaded dog. To appear in Nature.

60a. Erspamer, V. and Asero, B. Enteramine, the specific hormonic product of the entero-chromaffin system. Ricerca Sci., **21**: 2132–2136, 1951.

60b. Erspamer, V. and Ottolenghi, A. Antidiuretic action of enteramine. Experientia, **6**: 428, 1950.

60c. Erspamer, V. and Ottolenghi, A. Preliminary researches on the mechanism of the antidiuretic action of enteramine. Experientia, **7**: 191, 1951.

60d. Erspamer, V. and Ottolenghi, A. Antidiuretic action of small doses of enteramine in the rat. Experientia, **8**: 31, 1952.

61. Eversole, W. J. Effects of adrenal medullary hormones on blood and urinary chloride levels during water diuresis. Federation Proc., **10**: 41-42, 1951.

62. Eversole, W. J., Birnie, J. H. and Gaunt, R. Inactivation of posterior pituitary antidiuretic hormone by liver. Endocrinology, **45**: 378-382, 1949.

63. Farnsworth, E. B. Electrolyte partition in patients with oedema of various origins. Am. J. Med., **4**: 338-342, 1948.

64. Fee, A. R. Studies on water diuresis. Part II. J. Physiol., **68**: 305-312, 1929.

65. Fee, A. R. and Hemingway, A. The oxygen usage of the kidney. J. Physiol., **65**: 100-108, 1928.

66. Feldberg, W. and Vogt, M. Acetylcholine synthesis in different regions of the central nervous system. J. Physiol., **107**: 372-381, 1948.

67. Fisher, C., Ingram, W. R. and Ranson, S. W. Diabetes insipidus and the neuro-hormonal control of water balance. Edward Bros. Inc., Michigan, 1938.

68. Frank, R. T. Hormonal causes of premenstrual tension. Arch. Neurol. & Psychiat., **26**: 1053-1057, 1931.

69. Franklin, K. J., McGee, L. E. and Ullmann, E. A. Effects of severe asphyxia on the kidney and urine flow. J. Physiol., **112**: 43-53, 1951.

70. Frey, E. Maximale oder minimale Rückresorption in der Niere. Arch. f. exper. Path. u. Pharmakol., **165**: 621-651, 1932.

71. Frey, E. Schaltstelle des Blutstromes in der Niere und Hypophysenhinterlappenhormon. Arch. f. exper. Path. u. Pharmakol., **182**: 633-641, 1936.

72. Friedlander, M., Laskey, N. and Silbert, S. Effect of estrogenic substance on blood volume. Endocrinology, **20**: 329-332, 1936.

72a. Gaunt, R., Birnie, J. H. and Eversole, W. J. Adrenal cortex and water metabolism. Physiol. Rev., **29**: 281-310, 1949.

73. Gaunt, R., Liling, M. and Cordsen, M. The adrenal medulla in water diuresis and water intoxication. Endocrinology, **37**: 136-146, 1945.

74. Geiling, E. M. K. Paul Reed Lockwood Lecture, State University, Iowa. 1940. Quoted from Smith (207).

75. Gibson, R. B. and Martin, F. T. Administration of pituitary extract in a case of diabetes insipidus. Arch. Int. Med., **27**: 351-360, 1921.

76. Gilbert, A. and Lereboullet, P. Des urines retardées (opsiurie) dans les cirrhoses. Compt. rend. Soc. biol., **53**: 276-279, 1901.

77. Gillman, J. Nature of subjective reactions evoked in women by progesterone with special reference to the problem of premenstrual tension. J. Clin. Endocrinol., **2**: 157-160, 1942.

78. Gilman, A. and Goodman, L. S. The secretory response of the posterior pituitary to the need for water conservation. J. Physiol., **90**: 113-124, 1937.

79. Gilman, A. and Kidd, N. E. The antidiuretic activity of blood and its possible relation to histamine. J. Pharmacol. & Exper. Therap., **63**: 10, 1938.

80. Ginsburg, M. A method for the assay of antidiuretic activity. Brit. J. Pharmacol., **6**: 411-416, 1951.

81. Ginsburg, M. and Heller, H. Antidiuretic activities in plasma and serum. J. Physiol., **115**: 43-44P, 1951.

82. Glaubach, S. and Molitor, H. Untersuchungen über die hormonal diureseregelnde Tätigkeit der Leber. Arch. f. exper. Path. u. Pharmakol., **132**: 31-49, 1928.

83. Goodwin, W. E., Sloan, R. D. and Scott, W. W. The "Trueta" renal vascular "shunt". An experimental demonstration of neurovascular control of the renal circulation in the rabbit, cat, dog and man. J. Urol., **61**: 1010-1027, 1949.

84. Goodyer, A. V. N., Relman, A. S., Lawrason, F. D. and Epstein, F. H. Salt retention in cirrhosis of the liver. J. Clin. Endocrinol., **29**: 973-981, 1950.

85. Gopalan, C. Antidiuretic factor in urine of patients with nutritional oedema. Lancet, **1**: 304-306, 1950.

86. Govaerts, P. and Cambier, P. La diurèse consécutive a l'absorption d'eau par voie entérale et parentérale Bull. Acad. roy. méd. Belgique, **10**: 730-778, 1930.

87. Green, H. N. and Stoner, H. B. The effect of adenosine triphosphate on a water diuresis in man. J. Physiol., **103**: 28P, 1944.

88. Greenhill, J. P. and Freed, S. C. Electrolyte therapy of premenstrual distress. J. Am. M. A., **117**: 504-506, 1941.

89. Gunning, R. E. L. The effects of adrenin on the urine flow of anesthetized and unanesthetized dogs. Am. J. Physiol., **45**: 528-534, 1917.

90 Guthkelch, A. N. and Zuckerman, S. Concentration of the blood in relation to the menstrual cycle in monkeys. J. Physiol., **89**: 51-53P, 1937.

91. Ham, G. C. A comparison of pituitrin and antidiuretic substance in human urine and placenta. J. Clin. Investigation, **20**; 439, 1941.

92. Ham, G. C. and Landis, E. M. A comparison of pituitrin with antidiuretic substance found in human urine and placenta. J. Clin. Investigation, **21**: 455-470, 1942.

93. Ham, G. C. and Rosenfeld, M. Ultracentrifugation of antidiuretic, chloruretic and pressor factors of posterior pituitary extracts. Bull. Johns Hopkins Univ., **71**: 18-23, 1942.

94. Handovsky, H. and Samaan, A. Observations on the renal circulation and secretion in the dog, with special reference to the effect of pituitary (posterior lobe) extract. J. Physiol., **89**: 14-31, 1937.

95. Hare, K., Hickey, R. C. and Hare, R. S. The renal excretion of an antidiuretic substance in the dog. Am. J. Physiol., **134**: 240-244, 1941.

96. Hare, K., Melville, E. V., Chambers, G. H. and Hare, R. S. The assay of antidiuretic material in blood and urine. Endocrinology, **36**: 323–331, 1945.

97. Harris, G. W. Neural control of the pituitary gland. Physiol. Rev., **28**: 139–179, 1948.

98. Hart, P. D'A. and Verney, E. B. Observations on the rate of water-loss by man at rest. Clin. Sc. **1**: 367–396, 1934.

99. Hartmann, H., Ørskov, S. L. and Rein, H. Die Gefässreaktionen der Niere im Verlaufe allgemeiner Kreislauf-Regulationsvorgänge. Arch. ges. Physiol., **238**: 239–250, 1937.

100. Heller, H. The effect of the hydrogen-ion concentration on the stability of the antidiuretic and vasopressor activities of posterior pituitary extracts. J. Physiol., **96**: 337–347, 1939.

101. Heller, H. The posterior pituitary principles of a species of reptiles (Tropidonotus natrix) with some remarks on the comparative physiology of the posterior pituitary gland generally. J. Physiol., **101**: 317–326, 1942.

102. Heller, H. The effect of neurohypophyseal extracts on the water balance of lower vertebrates. Biol. Rev., **20**: 147–158, 1945.

103. Heller, H. Antidiuretic hormone in pituitary glands of newborn rats. J. Physiol., **106**: 28–32, 1947.

104. Heller, H. Antidiuretic substances. J. Pharm. & Pharmacol., **3**: 619–630, 1951.

105. Heller, H. and Smirk, F. H. Studies concerning the alimentary absorption of water and tissue hydration in relation to diuresis. J. Physiol., **76**: 283–302, 1932.

106. Heller, H. and Urban, F. F. The fate of the antidiuretic principle of posterior pituitary extract *in vivo* and *in vitro*. J. Physiol., **85**: 502–518, 1935.

107. Heller, H. and Zaimis, E. J. The antidiuretic and oxytocic hormones in the posterior pituitary gland of newborn infants and adults. J. Physiol., **109**: 162–169, 1949.

108. Hoff, E. C., Kell, J. F., Hastings, N., Gray, E. H. and Sholes, D. M. Acute renal cortical ischemia produced by stimulation of the pressor area of the cerebral cortex. Federation Proc., **8**: 76, 1949.

109. Hoff, E. C., Kell, J. F., Hastings, N., Sholes, D. M. and Gray, E. H. Vasomotor, cellular and functional changes produced in kidney by brain stimulation. J. Neurophysiol., **14**: 317–332, 1951.

110. Hoff, H., and Wermer, P. Über psychische Beeinflussung der Tätigkeit des Hypophysinhinterlappens. Arch. f. exper. Path. u. Pharmakol., **133**: 97–102, 1928.

111. Hobbes, A. D., Eversole, W. J. and Rock, M. Adrenal medullary hormones in water diuresis. Proc. Soc. Exper. Biol. & Med., **75**: 58–61, 1950.

112. Houck, C. R. Possible renal vascular shunt in dogs during intravenous epinephrine. Federation Proc., **9**: 63, 1950.

113. Houck, C. R., Bing, R. H., Craig, F. N. and Visscher, F. E. Renal hyperemia after the intravenous infusion of adenylic acid, adenosine, or adenosinetriphosphate in the dog. Am. J. Physiol., **153**: 159–168, 1948.

114. Huber, G. C. On the morphology of the renal tubules of vertebrates. Anat. Rec., **13**: 305–339, 1917.

115. Hughes-Jones, N. T., Pickering, G. W., Sanderson, P. H., Scarborough, H. and Vandenbrucke, J. The nature of the action of renin and hypertensin on renal function in the rabbit. J. Physiol., **109**: 288–307, 1949.

116. Hurwitz, D. and Bullock, L. T. Failure to find pressor and antidiuretic substances in patients with toxemia of pregnancy. Am. J. M. Sc., **189**: 613–619, 1935.

117. Ingram, W. R., Ladd, L. and Benbow, J. T. The excretion of antidiuretic substances by cats. Am. J. Physiol., **123**: 107, 1938.

118. Ingram, W. R., Ladd, L. and Benbow, J. T. Excretion of antidiuretic substance and its relation to hypothalamico-hypophyseal system in cats. Am. J. Physiol., **127**: 544–551, 1939.

119. Irving, G. W., Dyer, H. N. and du Vigneaud, V. Purification of the pressor principle of the pituitary gland by electrophoresis. J. Am. Chem. Soc., **63**: 503–506, 1941.

120. Janssen, S. Über zentrale Wasserregulation und Hypophysenantidiurese. Arch. f. exper. Path. u. Pharmacol., **135**: 1–18, 1928.

121. Jeffers, W. A., Livezey, M. M. and Austin, J. H. A method for demonstrating an antidiuretic action of minute amounts of pitressin; statistical analysis of results. Proc. Soc. Exper. Biol. & Med., **50**: 184–188, 1942.

122. Jones, A. M. and Schlapp, W. The action and fate of injected posterior pituitary extract in the decapitated cat. J. Physiol., **87**: 144–157, 1936.

123. Kamm, O., Grote, I. W. and Rowe, L. W. The possibility of interconversion of pituitary hormone and the formation of derived hormone from the β-hormone of the posterior lobe. J. Biol. Chem., **92**: 69–70, 1931.

124. Keele, C. A. and Slome, D. Adenosine triphosphate and renal blood flow. J. Physiol., **104**: 26–27P, 1945.

125. Keys, A. B. The heart-gill preparation of the eel and its perfusion for the study of a natural membrane *in situ*. Chloride and water secretion and absorption by the gills of the eel. Z. vergl. Physiol., **15**: 352–363; 364–388, 1931.

125a. Keys, A. and Willmer, E. N. "Chloride secreting cells" in the gills of fishes, with special reference to the common eel. J. Physiol., **76**: 368–377, 1932.

126. Klisiecki, A., Pickford, M., Rotschild, M. and Verney, E. B. The absorption and excretion of water by the mammal. Proc. Roy. Soc. B., **112**: 496–547, 1933.

127. Knowlton, F. P. and Silverman, A. C. The action of pituitary extract on the kidney. Am. J. Physiol., **47**: 1–12, 1918.

128. Kofford, H. Investigation of the kidneys of the kangaroo rat by the maceration method. Scandinav. J. Clin. & Lab. Med., **1**: 340, 1949.

129. v. Konschegg, A. and Schuster, E. Über die Beeinflussung der Diurese durch Hypophysin Extrakt. Deutsche med. Wchnschr., **41**: 1091–1095, 1915.

130. Korr, I. The osmotic function of the chicken kidney. J. Cell. & Comp. Physiol., **13**: 175–193, 1939.

131. Krieger, V. I. and Kilvington, T. B. Antidiuretic activity in the blood of nonpregnant women and women during normal and toxemic pregnancy. J. Clin. Endocrinol., **6**: 320–324, 1946.

132. Krohn, P. L. and Zuckerman, S. Water retention and skin swelling in pig-tailed macaque. J. Physiol., **88**: 369-387, 1937.

133. Kubicek, W. G., Harvey, R. B. and Kottke, F. J. The adrenaline sensitivity of the denervated dog kidney. Federation Proc., 7: 68, 1948.

134. Kuschinsky, G. and Liebert, P. Untersuchungen über den Hormongehalt des Hypophysenhinterlappens der Ratte unter dem Einfluss von Wasser, Kochsalz und Novasurol. Klin. Wchnschr., **18**: 823, 1939.

135. Kustner, H. Eigenartige Wirkung des Schwangerenserum besonders bei Eklampsie. Zbl. Gynaec., **51**: 2080-2081, 1927.

136. Labby, D. H. Studies of water storage in liver disease. III The antidiuretic activity of the urine during acute infective hepatitis. J. Clin. Investigation, **28**: 795, 1949.

137. Lampe, W. Die Beeinflussung der Diurese durch Leber Extrakte. Arch. f. exper. Path. u. Pharmakol., **119**: 83-90, 1926.

138. Lamport, H. The effect on renal resistance to blood flow of renin, angiotonin, pitressin, atropine, hypertensin, and toxemia of pregnancy. J. Clin. Investigation, **21**: 685-696, 1942.

139. Lauson, H., Eder, H. A., Chinard, F., Cotzias, G. C. and Greif, R. L. Estimation of the rate of antidiuretic hormone secretion in normal man. Federation Proc., 7: 69-70, 1948.

140. Leschke, E. Diabète insipide et systeme hypothalamo-hypophysaire. Ann. Med., **33**: 261-271, 1933.

141. Leslie, S. H., Johnson, B. and Ralli, E. P. Renal clearances in patients with cirrhosis of the liver, with and without ascites. J. Clin. Investigation, **9**: 682, 1949.

142. Leslie, S. H. and Ralli, E. P. The effect in rats of high fat diets on the renal excretion of water and antidiuretic substances. Endocrinology, **41**: 1-11, 1947.

143. Levitt, G. The problem of an antidiuretic substance in the blood of patients with eclampsia and other hypertensive disease. With observations on the spinal fluid. J. Clin. Investigation, **15**: 135-142, 1936.

144. Lewis, A. A. G. and Chalmers, T. M. A nicotine test for the investigation of diabetes insipidus. Clin. Sc.. **10**: 137-144, 1951.

145. Livingston, A. E. Further experiments on the effects of small doses of vasoconstrictor substances on the kidney. J. Pharmacol. & Exper. Therap., **32**: 181-188, 1928.

146. Ljungberg, E. On the reabsorption of chlorides in the kidney of the rabbit. Acta med. Scandinav., suppl., **127**: 7-189, 1947.

147. Luco, J. V. The defatiguing effect of adrenaline. Am. J. Physiol., **125**: 196-204, 1939.

148. McFarlane, A. The antidiuretic action of the pituitary. J. Pharmacol. & Exper. Therap., **28**: 177-208, 1926.

149. McIntyre, A. R. and van Dyke, H. B. The distribution and concentration of water and halides in blood and urine during diuresis-inhibition by pituitary extract. J. Pharmacol. & Exper. Therap., **42**: 155-168, 1932.

150. Mackersie, W. A. The diuretic and antidiuretic effect of pituitary extract and suggestions for a subsidiary test. J. Pharmacol. & Exper. Therap., **24**: 83-99, 1925.

151. McManus, M. A., Riley, G. A. and Janney, J. C. Kidney function in pregnancy. Am. J. Obst. & Gynec.. **28**: 524-533, 1934.

152. Maluf, N. S. R. Nephro-omentopexy, compensatory renal hyperfunction and measurements of renal dynamics. Am. J. Physiol., **156**: 79-86, 1949.

153. Marrazi, A. S. Electrical studies on the pharmacology of autonomic synapses; action of parasympathomimetic drugs on sympathetic ganglia. J. Pharmacol. & Exper. Therap., **65**: 395-404, 1939.

154. Martin, S. J., Herrlich, H. C. and Fazekas, J. F. Relation between electrolyte imbalance and excretion of antidiuretic substance in adrenalectomised cats. Am. J. Physiol., **127**: 51-57, 1939.

155. Marx, H. Untersuchungen über den Wasserhaushalt. II Mitteilung. Die psychische Beeinflussung des Wasserhaushaltes. Klin. Wchnschr., 5: 92-94, 1926.

156. Marx, H. Diuresis by conditioned reflex. Am. J. Physiol., **96**: 356-362, 1931.

157. Maxwell, M. H., Breed, E. S. and Smith, H. W. Significance of renal juxtamedullary circulation in man. Am. J. Med., **9**: 216-228, 1950.

158. Mazur, A. and Shorr, E. Hepato-renal factors in circulatory homeostasis. J. Biol. Chem., **176**: 771-787, 1948

159. Meier, H. Dei Beeinflussung der Harnabsonderung durch die Leber. Biochem. Ztschr., **209**: 200-217, 1929.

160. Merrill, A., Williams, R. H. and Harrison, T. R. The effects of a pressor substance obtained from the kidneys and the renal circulation of rats and dogs. Am. J. M. Sc., **196**: 240-246, 1938.

161. Molitor, H. and Pick, E. P. Zur Kenntniss der Diuresehemmung durch Hypophysenpreparate. Klin. Wchnschr., **2**: 2242-2243, 1923.

162. Molitor, H. and Pick, E. P. Über Diuresehemmung durch Histamin und Cholin. Arch. f. exper. Path. u. Pharmakol., 101: 198-206, 1924.

163. Molitor, H. and Pick, E. P. Über zentrale Regulation des Wasserwechsels. Arch. f. exper. Path. u. Pharmakol., **107**: 185-191, 1925.

164. Molitor, H. and Pick, E. P. Über zentrale Regulation des Wasserwechsels. Arch. f. exper. Path. u. Pharmakol., **112**: 113-121, 1926.

165. Motzfeldt, K. Experimental studies in the relationship of the pituitary body to renal function. J. Exper. Med., **25**: 153-188, 1917.

166. Nelson, E. E. and Woods, G. G. The diuretic and antidiuretic activity of posterior pituitary extracts. J. Pharmacol. & Exper. Therap., **50**: 241-253, 1934.

167. Noble, R. L., Rinderknecht, H. and Williams, P. C. Apparent augmentation of pituitary antidiuretic action by various retarding substances. J. Physiol., **96**: 293-301, 1939.

168. O'Connor, W. J. The effect of section of the supraoptico-hypophyseal tracts on the inhibition of water diuresis by emotional stress. Quart. J. Exper. Physiol., **33**: 149-162, 1945.

169. O'Connor, W. J. The control of urine secretion in mammals by the pars nervosa of the pituitary. Biol. Rev., **22**: 30-53, 1947.
170. O'Connor, W. J. and Verney, E. B. The effect of removal of the posterior lobe on the inhibition of water diuresis by emotional stress. Quart. J. Exper. Physiol., **31**: 393-408, 1942.
171. O'Connor, W. J. and Verney, E. B. The effect of increased activity of the sympathetic system in the inhibition of water diuresis by emotional stress. Quart. J. Exper. Physiol., **33**: 77-90, 1945.
172. Page, I. H. and Helmer, O. M. A crystalline pressor substance (angiotonin) resulting from the reaction between renin and renin activator. J. Exper. Med., **71**: 29-42, 1940.
173. Pénau, H. and Simonet, H. Titrage biologique de l'activité antidiuretique des extraits de lobe postèrior d'hypophyse. J. Pharm. Chim., Paris, **20**: 304-309, 1934.
174. Phillips, R. A., Dole, V. P., Hamilton, P. B., Emerson, K., Archibald, R. M. and van Slyke, D. D. The effects of acute hemorrhagic and traumatic shock on renal function of dogs. Am. J. Physiol., **145**: 313-336, 1946.
175. Pickering, G. W. and Prinzmetal, M. The effect of renin on urine formation. J. Physiol., **98**: 314-335, 1940.
176. Pickford, M. The inhibition of water diuresis by pituitary (posterior lobe) extract and its relation to the water load of the body. J. Physiol., **87**: 291-297, 1936.
177. Pickford, M. The inhibitory effect of acetylcholine on water diuresis in the dog, and its pituitary transmission. J. Physiol., **95**: 226-238, 1939.
178. Pickford, M. Control of the secretion of antidiuretic hormone of the pars nervosa of the pituitary gland. Physiol. Rev., **25**: 573-595, 1945.
179. Pickford, M. The action of acetylcholine in the supraoptic nucleus of the chloralosed dog. J. Physiol., **106**: 264-270, 1947.
180. Pickford, M. and Ritchie, A. E. Experiments on the hypothalamic-pituitary control of water excretion in dogs. J. Physiol., **104**: 105-128, 1945.
181. Pickford, M. and Watt, J. A. A comparison of the effect of intravenous and intracarotid injections of acetylcholine in the dog. J. Physiol., **114**: 333-335, 1951.
182. Pickford, M. and Watt, J. A. Comparison of some of the actions of adrenaline and noradrenaline on the kidney. Quart. J. Exper. Physiol., **36**: 205-212, 1951.
183. Potts, A. M. and Gallagher, T. F. Cystine, tyrosine and arginine content of high potency pressor and oxytocic pituitary hormone. J. Biol. Chem., **143**: 561-562, 1942.
184. Poulsson, L. T. Über die Wirkung des Pituitrins auf die Wasserauschiedung durch die Niere. Ztschr. f. d. ges. exper. Med., **71**: 577-620, 1930.
185. Ralli, E. P., Robson, J. S., Clarke, D. and Hoagland, C. L. Factors influencing ascites in patients with cirrhosis of the liver. J. Clin. Investigation, **24**: 316-325, 1945.
186. Rees, M. H. The influence of pituitary extracts on the absorption of water from the small intestine. Am. J. Physiol., **53**: 43-48, 1920.
187. Reubi, F. C. and Futcher, P. H. The effects of histamine on renal function in hypertensive and normotensive subjects. J. Clin. Investigation, **28**: 440-446, 1949.
188. Richards, A. N. and Plant, O. H. Urine formation in the perfused kidney. The influence of adrenaline on the perfused kidney. Am. J. Physiol., **59**: 144-183, 1922.
189. Richardson, J. A. and Houck, C. R. Renal tubular excretory mass and reabsorption of sodium, potassium and chloride in testosterone-treated and estradiol-treated female dogs. Federation Proc., **9**: 105-106, 1950.
190. Roboz, P. Über die Wirkung des Hypophysens auf den Wasserstoffwechsel. Arch. f. exper. Path. u. Pharmakol., **159**: 562-582, 1931.
191. Rosenfeld, M. The native hormones of the posterior pituitary gland: the pressor and oxytocic principles. Bull. Johns Hopkins Univ., **66**: 398-403, 1940.
192. Ross, H. and Stehle, R. L. On the immediate antidiuretic action of pituitary extract. J. Pharmacol. & Exper. Therap., **38**: 451-460, 1930.
193. Rydin, H. and Verney, E. B. The inhibition of water diuresis by emotional stress and muscular exercise. Quart. J. Exper. Physiol., **27**: 343-374, 1938.
194. Samaan, A. The effect of pituitary (posterior lobe) extract upon urinary flow in non-anaesthetized dogs. J. Physiol., **85**: 37-46, 1935.
195. Schaffer N. E., Cadden, J. F. and Stander, H. J. Measurement of antidiuretic activity as applied to eclamptic urine and properties of antidiuretic substances in rat urine, pituitary and beef liver. Endocrinology, **28**: 701-706, 1941.
196. Schlieper, C. Über die osmoreregulationische Funktion der Aal Kiemen. Z. vergl. Physiol., **18**: 682-695, 1933
197. Schmidt-Nielsen, B. and Schmidt-Nielsen, K. Reduced evaporative water loss from the lungs of certain desert mammals. Federation Proc., **8**: 139-140, 1949.
198. Schokaert, J. A. and Lambillon, J. Observations complémentaires sur la substance antagoniste de la vasopressine dans le sèrum de la femme enceinte. Compt. rend. Soc. biol., **122**: 478-480, 1936.
199. Shannon, J. A. The control of renal excretion of water. I. The effect of variations in the state of hydration on water excretion in the dog with diabetes insipidus. J. Exper. Med., **76**: 387-399, 1942.
200. Shapiro, B. B. The influence of follicular hormone on urinary secretion in man. J. Physiol., **92**: 3P, 1938.
201. Shorr, E., Zweichfach, B. W., Furchgott, R. F. and Baez, S. Hepato-renal factors in circulatory homeostasis. iv. Tissues origins of the vasotropic principles, VEM and VDM, which appear during evolution of hemorrhagic and tourniquet shock. Circulation, **3**: 42-79, 1951.
202. Silvette, H. Some effects of low barometric pressure on kidney function in the white rat. Am. J. Physiol., **140**: 374-386, 1943.

203. Simon, A. The pressor and oxytocic content of the hypophysis of rats under various conditions. Am. J. Physiol., **107:** 220–226, 1934.

204. Simon, A. and Kardos, Z. Über den Gehalt Hypophysinhinterlappen normaler und durstender Tiere an blutdruck- und uterus-wirksamer Stoffen. Arch. f. exper. Path. u. Pharmakol., **176:** 238–242, 1934.

205. Simon, A. and Nagy, F. Über den Gehalt menschlicher Hypophysinhinterlappen an blutdruck und uterus-wirksamer Stoffen. Arch. f. exper. Path. u. Pharmakol., **176:** 242–247, 1934.

206. Sinclair-Smith, B. C., Sisson, J., Kattus, A. A., Genecin, A., Monge, C., McKeever, W. and Newman, E. V. The effects of posterior pituitary extract and smoking on water, sodium and chloride excretion in normal subjects and in patients with congestive heart failure. Bull. Johns Hopkins Univ., **87:** 221–254, 1950.

207. Smith, H. W. The kidney. Oxford Univ. Press, Oxford, 1951.

208. Sperber, I. Studies in the mammalian kidney. Zool. Bidr., Upsala, **22:** 249–432, 1944.

209. Springorum, P. W. and Centenera, D. Die verschiedene Beteiligung beider Nieren an Diurese-änderungen und vasomotorischen Reaktionen. Arch. ges. Physiol., **239:** 440–450, 1937.

210. Starling, E. H. and Verney, E. B. The secretion of urine as studied in the isolated kidney. Proc. Roy. Soc., B., **97:** 321–363, 1925.

211. Steggerda, F. G. and Essex, H. E. Comparative study of effects of preparations of posterior lobe of pituitary gland on water interchange in frogs. Proc. Soc. Exper. Biol. & Med., **32:** 425–428, 1934.

212. Stehle, R. L. The chemistry of the posterior lobe of the pituitary gland. Vitamins and Hormones, **7:** 383–388, 1949.

213. Stehle, R. L. The actions of the hormone of the posterior lobe of the pituitary gland upon the circulation, and the secretion of urine. Vitamins and Hormones, **7:** 389–435, 1949.

214. Stehle, R. L. and Fraser, A. M. The purification of the pressor and oxytocic hormones of the pituitary gland and some observations on the chemistry of the products. J. Pharmacol. & Exper. Med., **55:** 136–151, 1935.

215. Stickney, J. C., Northrup, D. W. and van Liere, E. J. The effect of anoxic anoxia on urine secretion in anesthetized dogs. Am. J. Physiol., **147:** 616–621, 1946.

216. Study, R. S. and Shipley, R. E. Effects of renal artery-nerve stimulation on renal blood flow measured directly, and by clearance and extraction of diodrast. Am. J. Physiol,, **159:** 592, 1949.

217. Stueck, G. H., Leslie, S. H. and Ralli, E. P. Preliminary observations on the antidiuretic substance recovered from the urines of patients with cirrhosis of the liver. Endocrinology, **44:** 325–332, 1949.

218. Taylor, H. C., Warner, R. C. and Welsh, C. A. The relationship of the estrogens and progesterone to the edema of normal and toxemic pregnancy. Am. J. Obst. & Gynec., **45:** 547–568, 1943.

219. Taylor, N. B. G. and Walker, J. M. Antidiuretic substances in human urine after smoking. J. Physiol., **113:** 412–418, 1951.

220. Teel, H. M. and Reid, D. E. Observations upon the occurrence of an antidiuretic substance in the urine of patients with pre-eclampsia and eclampsia. Endocrinology, **24:** 297–310, 1939.

221. Theobald, G. W. The alleged relation of hyperfunction of the posterior lobe of the hypophysis to eclampsia and the nephropathy of pregnancy. Clin. Sc., **1:** 225–239, 1934.

222. Theobald, G. W. The repetition of certain experiments on which Molitor and Pick base their water-centre hypothesis, and the effect of afferent nerve stimuli on water diuresis. J. Physiol., **84:** 243–254, 1934.

223. Theobald, G. W. and Verney, E. B. The inhibition of water diuresis by afferent nerve stimuli after complete denervation of the kidney. J. Physiol., **83:** 341–351, 1935.

224. Theobald, G. W. and White, M. Antidiuretic substances from liver. J. Physiol., **78:** 18P, 1933.

225. Thomas, W. A. Generalised edema occurring only at the menstrual period. J. Am. M. A., **101:** 1126–1127, 1933.

226. Thorn, G. W. and Emerson, K. The role of gonadal and adrenal cortical hormones in the production of edema. Ann. Int. Med., **14:** 757–766, 1940.

227. Thorn, G. W. and Engel, L. L. The effect of sex hormones on the renal excretion of electrolytes. J. Exper. Med., **68:** 299–312, 1938.

228 Thorn, G.W., Nelson, K. R. and Thorn, D. W. A study of the mechanism of edema associated with menstruation. Endocrinology, **22:** 155–163, 1938.

229. Toth, L. A. The effects of epinephrine on urine secretion in the dog. Am. J. Physiol., **119:** 140–148, 1937.

230. Toth, L. A. Urine excretion during anoxia of normal and denervated kidneys in the dog with and without adrenal glands. Am. J. Physiol., **129:** 532–538, 1940.

231. Trueta, J., Barclay, A. E., Franklin, K. J., Daniel, P. M. and Prichard, M. M. L. Studies of the renal circulation. Blackwell Scientific Publications, Oxford, 1947.

232. Underhill, F. P. and Pack, G. T. The influence of various diuretics on the concentration of the blood. Am. J. Physiol., **66:** 520–552, 1923.

234. Unna, K. Arterieller Druck und Nieren-durchblutung. Arch. ges. Physiol., **235:** 515–519, 1935.

235. Vaichulis, J. A. Effect of autolysis on differential solubility of principles of posterior lobe of pituitary body. Endocrinology, **32:** 361–366, 1943.

236. van Dyke, H. B., Chow, B. F., Greep, R. O. and Rothen, A. The isolation of a protein from the pars neuralis of the ox pituitary with constant pressor, oxytocic and diuresis-inhibiting activities. J. Pharmacol. & Exper Therap., **74:** 190–209, 1942.

237. Veil, W. H. Uber intermediäre Vorgänge beim Diabetes insipidus und ihre Bedeutung für die Kenntniss vom Wesen diese Leidens. Biochem. Ztschr., **91:** 317–380, 1918.

238. Verney, E. B. Secretion of pituitrin in mammals as shown by perfusion of the isolated kidney of the dog. Proc. Roy. Soc., B., **99:** 487–517, 1926.

239. Verney, E. B. Absorption and excretion of water; antidiuretic hormone. Lancet, 739–744; 781–783, 1946.

240. Verney, E. B. The antidiuretic hormone and the factors which determine its release. Proc. Roy. Soc., B., **135:** 25–106, 1947.

241. Verney, E. B. and Vogt, M. Observations on the effect of renal ischaemia upon arterial pressure and urine flow in the dog. Quart. J. Exper. Physiol., **32:** 35–65, 1943.

242. du Vigneaud, V., Irving, G. W., Dyer, H. M. and Sealock, R. R. Electrophoresis of posterior pituitary gland preparations. J. Biol. Chem., **123:** 45–55, 1938.

243. Vogt, M. Communication to the British Pharmacological Society, January, 1952.

244. Wakim, K. G., Herrick, J. F., Baldes, J. and Mann, F. C. The effect of pitressin on renal circulation and urine secretion. J. Clin. & Lab. Med., **27:** 1013–1022, 1942.

245. Walker, A. M. An antidiuretic effect produced by certain preparations of heparin. Proc. Soc. Exper. Biol. & Med., **39:** 105–106, 1938.

246. Walker, A. M. Experiments upon the relation between pituitary gland and water diuresis. Am. J. Physiol., **127:** 519–540, 1939.

247. Walker, A. M., Bott, P. A., Oliver, J. and McDowell, M. C., The collection and analysis of fluid from single nephrons of the mammalian kidney. Am. J. Physiol., **134:** 580–595, 1941.

248. Walker, A. M., Schmidt, C. F., Elsom, K. A. and Johnston, C. G. Renal blood flow of unanesthetized rabbits and dogs in diuresis and antidiuresis. Am. J. Physiol., **118:** 95–110, 1937.

249. Watson, C. J. and Greenberg, A. Certain effects of salt-poor human albumin in cases of hepatic disease. Am. J. M. Sc., **217:** 651–657, 1949.

250. Weir, T. F., Larson, E. E. and Rowntree, L. G. Studies in diabetes insipidus, water balance, and water intoxication. Arch. Int. Med., **29:** 306–309, 1922.

251. Welsh, C. A., Wellen, I. and Taylor, H. C. The filtration rate, effective renal blood flow, tubular excretory mass and phenol red clearance in normal pregnancy. J. Clin. Investigation, **21:** 57–62, 1942.

252. Wesson, L. G., Anslow, W. P. and Smith, H. W. The excretion of strong electrolytes. Bull. New York Acad. Med., **24:** 586–606, 1948.

253. White, H. L., Heinbecker, P. and Rolf, D. Some endocrine influences on renal function. Am. J. Physiol., **149:** 404–417, 1947.

254. Winton, F. R. The control of the glomerular pressure by vascular changes within the isolated mammalian kidney, demonstrated by the action of adrenaline. J. Physiol., **73:** 151–162, 1931.

255. Winton, F. R. Physical factors involved in the activity of the mammalian kidney. Physiol. Rev., **17:** 408–435, 1937.

256. Wirz, H., Hargitay, B. and Kuhn, W. Lokalisation der Konzentrierungsprozessus in der Niere durch direkte Kryoskopie. Helv. Physiol. Acta., **9:** 196–207, 1951.

257. Wirz, H. Personal communication.

258. Witten, C. M. and Bradbury, J. T. Hemodilution as a result of oestrogen therapy. Proc. Soc. Exper. Biol. & Med., **78:** 626–629, 1951.

259. Yanagi, K. The effect of posterior pituitary preparations upon the colloid osmotic pressure of serum protein, water and mineral metabolism of dogs. J. Pharmacol. & Exper. Therap., **56:** 23–38, 1936.

260. Zuckerman, S., Palmer, A. and Hanson, D. A. The effect of steroid hormones on the water content of the tissues. J. Endocrinol., **6:** 261–276, 1950.

QUANTITATIVE ASPECTS OF IODINE METABOLISM IN MAN*

DOUGLAS S. RIGGS

Department of Pharmacology, Harvard Medical School, Boston, Mass.

TABLE OF CONTENTS

I. Introduction 285
II. Quantitative Methods Available for the Study of Iodine Metabolism in Man 286
  A. Chemical Methods 286
  B. Radioactive Iodine 286
III. Normal Pathways of Iodine Metabolism 288
  A. General Description 288
  B. Simplifying Assumptions 290
    1. Compartments 290
      (a) Inorganic Iodide 290
      (b) Organic Iodine in the Thyroid Gland 291
      (c) Organic Iodine in the Blood and Extrathyroidal Tissues 291
    2. Metabolic Pathways 291
    3. Assumptions Concerning the Distribution of a Tracer Dose of Radioactive Iodine 292
IV. Equilibrium States and Iodine Balance 294
V. Symbols, Dimensions, Units and Definitions 296
VI. Factors which Influence the Activity of the Thyroid Gland 298
  A. The Renal Clearance of Iodide 301
  B. The Rate of Secretion of the Thyroid Hormone 303
    1. Methods Based on Substitution Therapy 303
    2. Methods Based on the Uptake of Radioactive Iodine by the Gland and the Excretion of Stable Iodine in the Urine 305
    3. Methods Based on Specific Activity 307
    4. Other Methods 309
  C. The Daily Intake of Iodine 310
  D. The Excretion of Organic Iodine 310
    1. Urine 311
    2. Feces 313
  E. The Blood Flow through the Thyroid Gland 317
  F. The Efficiency of Extraction of Iodide from the Blood Stream 318
VII. Factors which Influence the Distribution of Radioactive Iodine 320
  A. The Iodide Compartment 321
  B. Organic Iodine in the Thyroid Gland 324
  C. Organic Iodine in the Extrathyroidal Tissues 327
VIII. Physiological Variations in Iodine Metabolism and Thyroid Function 329
  A. Sex 331
  B. Age 331
  C. Pregnancy 331
  D. Exposure to Cold 332
  E. Other Stresses 332
  F. Iodine Deficiency 332
  G. Other Dietary Factors 335

---

* The preparation of this review and some of the original work reported in it have been supported by grants from the Eugene Higgins Trust and from the United States Public Health Service.

IX. Pathological Variations in Iodine Metabolism and Thyroid Function 335
  A. Hypothyroidism 335
  B. Hyperthyroidism 336
  C. Sporadic Cretinism 340
X. The Influence of Drugs on Iodine Metabolism and Thyroid Function 341
  A. Exogenous Thyroid Hormone 341
  B. Iodide 342
  C. Thiocyanate and Other Anions 344
  D. Goitrogenic Drugs of the Thioamide Series 345
XI. Diagnostic Tests 347
  A. The Concentration of Protein-bound Iodine in the Plasma 348
  B. The Uptake of Radioactive Iodine by the Thyroid Gland 353
  C. The Clearance of Iodide by the Thyroid Gland 356
  D. The Rate of Accumulation of Iodide by the Thyroid Gland 356
  E. The Conversion Ratio 359
XII. Conclusion 363

I. INTRODUCTION

During the past fifteen years three developments have enormously facilitated the quantitative study of iodine metabolism and thyroid function. The first of these has been the production of radioactive isotopes of iodine. The second has been the gradual perfection of accurate methods for the separation, identification and chemical determination of the minute amounts of iodine-containing compounds encountered in biological materials. The third has been the discovery of pharmacological agents which, by interfering with certain specific steps in the manufacture of thyroid hormone, make possible the biochemical dissection of thyroid function in living animals. Much of the new information which has accrued from the use of these physical, chemical, and pharmacological tools has already been reviewed by others. Excellent discussions of the steps in the synthesis of thyroxine by the thyroid gland (70, 71), the mechanism of action of goitrogenic drugs (7, 8, 27, 133), the occurrence of thyroid-inhibiting compounds in food (58), and the mode of action of the thyroid hormone in tissues (12) have recently been published. In addition, several authors have presented well-balanced appraisals of the clinical value of the various methods currently available for the diagnosis (81, 107, 137, 175), and treatment (7, 32, 106, 107, 108, 110, 177) of thyroid disease. These topics will be mentioned here only briefly or not at all.

The present review is an attempt to describe, as precisely as present information will permit, all the factors which influence iodine metabolism and hence the activity of the thyroid gland. Although the results of animal experiments will sometimes be mentioned, most of the discussion will be concerned with man. The scope of this review is further restricted by deliberate neglect of the mechanism of hormone secretion (42), and the metabolism of synthetic iodine-containing compounds which are foreign to the body. Besides these restrictions, the author must confess that his review of the literature has been highly selective, rather than comprehensive, and that he may have inadvertently overlooked important papers. Fortunately much of the recent work on iodine metabolism, including information derived from animal experiments, has been very well summarized by Albert (1), and his extensive bibliography contains a number of pertinent references not included in the present paper. It is hoped that in spite of these deficiencies the review will be useful as an illustration of how the direct measurement of a few variables can be combined with simple mathematical deductions so as to provide a reasonably complete and detailed *quantitative* description of the metabolic pathways followed by a chemical element in man.

II. QUANTITATIVE METHODS AVAILABLE FOR THE STUDY OF IODINE METABOLISM IN MAN

A. *Chemical Methods:* The ability of traces of the iodide ion to catalyze the reaction between ceric salts and arsenious acid was first described by Sandell and Kolthoff (170). Chaney adapted this reaction to the analysis of iodine in biological materials (25), and many exquisitely sensitive modifications of the Chaney method are now in widespread use (11, 22, 31, 97, 186). With this method it is possible to detect quantities of iodine as small as 0.005 microgm. Somewhat larger quantities may be measured with considerable precision so that one or two ml. of plasma or serum suffice for a single analysis. The analytical technique is also far less time-consuming and far more objective than previous methods. However, except for occasional analyses of iodine in lymph, cerebrospinal fluid or edema fluid, most analysts have restricted themselves to serum, urine or samples of the thyroid gland removed at operation or autopsy. Few recent studies have dealt with the iodine content of food, feces or sweat.

B. *Radioactive Iodine:* The advantages of radioactive iodine as a tool for the investigation of iodine metabolism and thyroid function have been adequately described by others (80, 83, 141, 149, 150, 175) and need not be reiterated here. The isotope I^{131} with a half life of 8.0 days is made available as iodide from the uranium pile at Oak Ridge to investigators with proper facilities for its safe handling. Since it is available practically uncontaminated with inert iodine ("carrier-free") it can be given either by mouth or by vein without causing any detectable increase in the total quantity of iodine in the body. It can thus be used to measure the dynamic behavior of iodide without upsetting normal equilibrium. Much of the usefulness of radioactive iodine lies in the possibility of measuring it not only in samples of body fluids and urine, but also *in vivo* as it accumulates in the thyroid gland. A discussion of the precautions required for accurate measurements of radioactivity in the thyroid gland lies outside the scope of this paper. Suffice it to say that due consideration must be given to the elimination of radiation coming from other parts of the body, to the effect of the surrounding neck tissues on radiation emanating from the gland, and to the geometrical relationship between the gland itself and the counting apparatus (82, 120, 127).

The techniques of butanol extraction and chromatographic separation have been used to good advantage in the separation and identification of the various compounds into which radioactive iodine becomes incorporated during its sojourn in the body. These preparative techniques are often facilitated by the addition of comparatively large quantities of the iodine-containing compounds under scrutiny, the added "carrier" compounds containing only I^{127}. When carriers are used, the conditions of extraction and purification must preclude exchange reactions between the stable and radioactive isotopes (89, 112).

Two practical considerations limit the quantity of radioactivity which can be administered for physiological studies in the human. While in certain patients with hyperthyroidism or thyroid carcinoma amounts of radioactive iodine sufficiently large to damage the thyroid gland are purposely administered, it should be obvious that under these circumstances the fate of the radioactive iodine cannot provide reliable information about the *normal* physiology of the thyroid gland. During the first few days after the administration of such a "therapeutic" dose, the damaged thyroid parenchyma may occasionally permit large quantities of preformed hormone to leak out into the blood stream (155, 163). Not only will this cause an elevation in the concentration of serum protein-bound iodine, but there is also evidence that some of the material thus gaining access to the circulation is actually thyroglobulin (152, 193) which under normal circumstances is never secreted by the thyroid gland (91). Later, the diminution in thyroid activity caused by radiation damage will continue to upset the equilibrium which was presumably present at the time of administration of the radioactive iodine. For physiological studies it is therefore necessary to limit the dose to one which will surely not produce any detectable alteration in thyroid function. Although estimates vary, it seems probable that single doses of 100 microcuries or less will comply with this requirement.

The second limitation on the quantity of radioactive iodine which may legitimately be administered to man can neither be easily defined nor lightly dismissed (20, 32, 125). It is well recognized that exposure of a tissue to ionizing radiation may eventually produce malignant degeneration, often years after the initial exposure (45). While no instances of carcinogenesis due to the administration of radioactive iodine to man have as yet been reported, the production of thyroid carcinomas in rats given large doses of radioactive iodine has been clearly demonstrated by Goldberg and Chaikoff (50). Until enough time has elapsed to permit a final evaluation of the late effects of radioactive iodine in humans, every investigator is morally obliged to limit the quantity of radioactive iodine administered for physiological studies to the smallest amount which will suffice for the purpose at hand. This is of particular importance when repeated administration of tracer doses to the same individual is contemplated, since there is evidence that the effects of exposure to radiation are cumulative (20, 118).

These restrictions on the quantity of radioactive iodine which may be legitimately employed for physiological studies limit the time after a tracer dose during which reliable measurements of radioactivity can be made. In long-term studies not only is radioactive iodine lost through various excretory channels, but also the physical decay inexorably reduces the quantity of radioactive iodine remaining in the body. Thus, even if there were no excretion of iodine, a study involving serial measurements for a period of a month after a single tracer dose would require the administration of about fifteen times as much radioactive iodine as would suffice for a brief study of comparable precision. Fortunately, the development of more sensitive recording devices such as the scintillation counter has considerably extended the time during which useful measurements can be made after the administration of a tracer dose of radioactive iodine (5, 19).

A sharp distinction must be drawn between the kind of information obtained with tracer doses of radioactive iodine and the kind of information obtained with chemical methods of analysis. Tracer studies are ideal for the measurement of the *proportion* of the iodine in the body which follows a particular metabolic pathway and for the study of the *rate of turnover* of iodine within the various compartments. By themselves, however, tracer studies cannot indicate the actual amounts of iodine which are being metabolized. Chemical methods on the other hand give valuable information concerning the *quantities* of iodine with which the body deals, but by themselves provide no more than a dim outline of the dynamics of iodine metabolism. Only by the combined use of radioactive tracers and of chemical methods can a clear picture of the over-all metabolism of iodine be obtained.

III. NORMAL PATHWAYS OF IODINE METABOLISM

A. *General Description:* In the interests of clarity it is well to begin with a simple qualitative description of the various routes normally traversed by iodine entering the body. These routes are diagrammatically portrayed in Figure 1.

Iodide ingested with food and water is rapidly absorbed from the gastrointestinal tract into the blood stream. While some absorption may occur from the stomach (78), the majority is absorbed by the intestine. If administered as free iodine or iodate, reduction to iodide must occur in the intestine before absorption can take place (30). From the blood stream the iodide rapidly diffuses into the extracellular fluid of the tissues, its distribution for the most part being very similar to that of chloride (199). Although largely excluded from most cells, the iodide ion freely traverses the red blood cell membrane. The concentration of iodide in the water of red blood cells is approximately equal to its concentration in the water of plasma, so that a given volume of red blood cells contains about 65 per cent as much iodide as an equal volume of plasma (119, 144, 157). Distribution equilibrium between red blood cells and plasma is attained with great rapidity (119, 159).

In certain important respects, however, the distribution of the iodide ion differs significantly from the distribution of the chloride ion, particularly when the concentration of iodide is small. The most obvious of these differences is the accumulation of the iodide ion in the thyroid gland (see Section X, C). In addition, the concentration of iodide in salivary and gastric secretions far exceeds its concentration in extracellular fluid (119, 99). The thirty-fold increase in concentration achieved by the salivary and gastric glands is of the same order of magnitude as the concentration gradient which can be maintained by the normal thyroid gland. This similarity is particularly interesting since phylogenetically and embryologically the thyroid gland is derived from the anterior portion of the primitive gut (51). It would be interesting to determine whether thyrotropic hormone increases the ability of the salivary glands and gastric mucosa to concentrate the iodide ion as it increases the concentrating capacity of the thyroid gland. As pointed out by Myant and his co-workers (119), the very high concentrations of iodide in saliva and gastric juice may account in part for the fact that the iodide ion has a larger volume of distribution than the chloride ion.

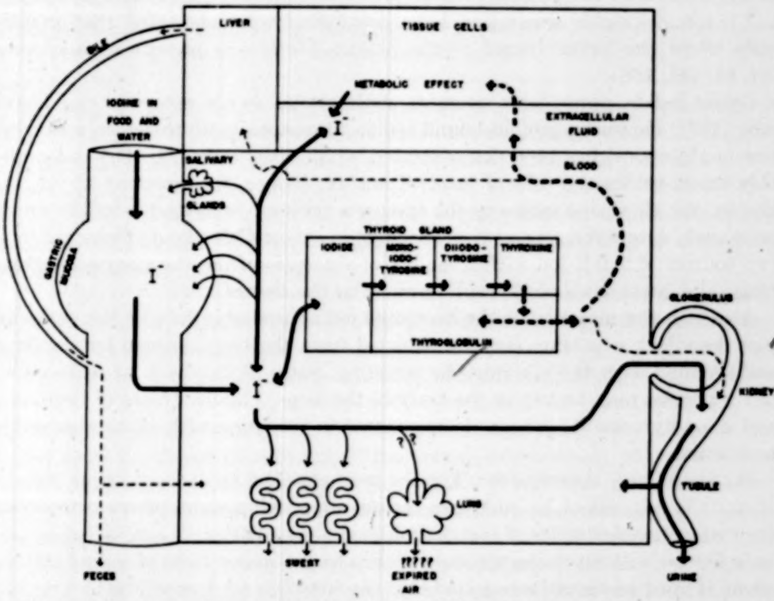
LIVER
TISSUE CELLS
BILE
METABOLIC EFFECT
IODINE IN FOOD AND WATER
I^-
EXTRACELLULAR FLUID
SALIVARY GLANDS
BLOOD
GASTRIC MUCOSA
I^-
THYROID GLAND
IODIDE
MONO-IODO-TYROSINE
DIIODO-TYROSINE
THYROXINE
GLOMERULUS
THYROGLOBULIN
I^-
KIDNEY
??
TUBULE
LUNG
FECES
SWEAT
????? EXPIRED AIR
URINE

FIG. 1. A diagrammatic portrayal of iodine metabolism. Major pathways are represented by thick arrows, minor pathways by thin arrows. Solid lines designate inorganic, and broken lines organic iodine. It is obvious that the complex scheme depicted in this figure must be considerably simplified for convenient mathematical description.

Although small quantities of iodide may be lost from the body in sweat, feces and possibly expired air, the vast majority of the iodide in extracellular fluid eventually follows one of two pathways. It is either trapped by the thyroid gland to be manufactured into thyroid hormone, or excreted by the kidney. The thyroid gland and the kidney are therefore in competition with each other for the available supply of iodide. In the kidney the iodide is filtered through the glomeruli and is then partially reabsorbed by the tubules but to a far smaller extent than is chloride, at least in the human (see Section VI, A).

Once trapped by the thyroid, iodide is rapidly converted to organic iodine by oxidation and combination with tyrosine. In successive reactions monoiodotyrosine, diiodotyrosine and thyroxine are synthesized, and the completed hormone is pooled with the preformed hormone already stored in the gland as thyroglobulin.[1] Under the controlling influence of the thyrotropic hormone of the anterior pituitary gland the thyroid hormone is secreted into the blood stream in whatever amount is needed to maintain a reasonably constant plasma concentration. Although the thyroid hormone may leave the gland as free thyroxine (90), upon entering the blood stream it becomes rapidly, firmly and almost completely bound to the plasma proteins. Several studies with butanol extraction and chromatographic separation have conclusively demonstrated that practically all of the iodine bound to the plasma proteins is present as thyroxine (64, 88, 161, 188).

Unlike iodide, appreciable amounts of thyroxine do not enter the red blood cells (157). However, protein-bound iodine, presumably thyroxine or a derivative of thyroxine, has been demonstrated within liver cells (23, 109) and probably exists within the cells of most, if not all, tissues. By reactions as yet unknown, the thyroxine iodine in the tissues is reduced to inorganic iodide which once again enters the general pool of iodide in extracellular fluid. There are thus two sources of iodide ion within the body: absorption from the gastro-intestinal tract, and breakdown of thyroid hormone in the tissues.

Although the majority of the hormonal iodine probably follows the pathway just described, a portion may be removed from the blood stream by the liver and secreted with the bile into the intestine. Some of this may be reabsorbed, but a portion may be lost to the body in the feces. Finally, traces of thyroxine and diiodotyrosine have been demonstrated in the urine with chromatographic techniques.

B. *Simplifying Assumptions:* For the mathematical analysis of iodine metabolism it is convenient to introduce certain simplifying assumptions concerning the rather complex series of events described above. Although simplification can be achieved without doing serious violence to the major facts of iodine metabolism, it must be clearly recognized that the resulting scheme will be to a certain extent abstract and artificial. The employment of such an abstract model for a complicated biological system is warranted only if each assumption is explicitly stated and justified. This will be done in the following paragraphs.

1. *Compartments:* It will be assumed that all of the iodine in the body is divided into three separate compartments.

(a) *Inorganic Iodide:* This compartment contains all of the inorganic iodide in the body including the iodide in the secretions of the alimentary tract, the iodide in red blood cells, and the iodide which is present in the thyroid gland and which has not yet been incorporated into protein. Although Oddie (126) has chosen to treat the inorganic iodide in the thyroid gland as a separate compartment, there seems to be no compelling reason to do so, since there is good evidence that iodide ions within the thyroid are freely exchangeable with the

---

[1] Note that in Figure 1 the transformation of iodide to thyroxine is depicted as a "one-way street". The reviewer once suggested that the thyroid gland might be capable of degrading exogenous hormone to inorganic iodide (158). He now believes, in complete agreement with Greer (59), that this is highly improbable.

iodide ions in the blood stream. One practical difficulty does arise from inclusion of the iodide within the thyroid gland as a segment of the general iodide compartment. During studies with radioactive iodine, radiation emanating from iodide ions within the thyroid will necessarily be measured together with radiation from iodine which has already become organically bound, and may therefore erroneously be ascribed to the compartment of organic iodine in the thyroid gland. Usually the transformation of inorganic to organic iodine is so rapid that this error will be negligible. However, if conversion to organic iodine is blocked, due allowance must be made for the presence in the thyroid gland of a considerable amount of radioactivity which properly belongs to the iodide compartment.

(b) *Organic Iodine in the Thyroid Gland:* To this compartment are assigned all of the organic compounds of iodine which occur in the thyroid. No distinction is made between iodine present as monoiodotyrosine, diiodotyrosine, thyroxine, or any unidentified compounds which may also be present (64, 160, 189). Disregard of the separate molecular species actually present is justified by the impossibility of measuring the transformation of one to another in the intact human being.

(c) *Organic Iodine in the Blood and Extrathyroidal Tissues:* This compartment contains all of the thyroid hormone outside of the thyroid gland including any thyroxine which is undergoing enterohepatic circulation.

It should be obvious that these so-called compartments do not exist within the body as actual physical entities with clearly defined boundaries, but are merely convenient abstractions. Within each compartment the iodine is assumed to be uniformly distributed. If the actual concentration of iodine in one region of the body is relatively high, that region will represent a relatively large portion of the compartment. This may lead to occasional difficulties in interpretation. For example, Myant and his collaborators (119) have shown that after the administration of a tracer dose of radioactive iodine its concentration in saliva or in gastric juice depends upon the concentration in plasma. However there is a delay of approximately thirty minutes between the attainment of peak concentration in the plasma and peak concentration in the secretions of the alimentary tract. The authors point out that the thirty-minute lag may account in part for the seeming expansion of the iodide compartment which they have noted in human subjects. Fortunately, this delay in the attainment of equilibrium is not likely to interfere seriously with the interpretation of observations which extend over a period of more than a few hours.

2. *Metabolic Pathways:* The assignment of all of the iodine of the body to three compartments automatically eliminates from consideration certain of the metabolic pathways illustrated in Figure 1. Still further simplification is warranted. While significant quantities of iodide may be lost in the *sweat* during profuse perspiration (178), this avenue of excretion is usually negligible and will not be considered in the discussion which follows. Moreover iodide is so efficiently absorbed by the intestine that only minute traces of iodide ion escape from the body in the *feces* (80, 124). Salter has placed considerable emphasis on the so-called "pulmonary clearance" of iodide, and apparently believes that

very considerable quantities of iodide can escape with water vapor from the lungs and be lost to the body in the *exhaled air* (169). He cites one study in which, 48 hours after the administration of a large therapeutic dose of radioactive iodine to a patient with thyroid cancer, slightly less than 700 microcuries of radioactive iodine remained in the body. At that time the patient was said to exhale almost 2 microcuries per breath. Allowing 20 breaths per minute, this would indicate that iodide was being excreted in the expired air at the patently preposterous rate of about 5 per cent of the amount remaining in the body per minute. In the same paper he later more conservatively estimates that nearly 0.3 per cent of the body's reserve of iodide may be exhaled per hour. The reviewer cannot believe that conditions favorable to the volatilization of iodide are at all likely to occur in the pulmonary epithelium and he is inclined to ascribe Salter's findings to technical error. This belief is strengthened by the fact that Keating was able to find only minute traces of radioactivity in the expired air of patients who had received therapeutic doses of radioactive iodine. The amount recovered was of the order of 0.1 per cent of the dose per 24 hours, and Keating thinks that even this small quantity may have been due to inadvertent contamination with droplets of saliva (79). In the following discussion no further mention will be made of the pulmonary excretion of iodide.

For purposes of mathematical treatment one further simplification will be introduced. The organic iodine excreted in feces and urine will usually be considered as a single entity. The only justification for so doing is that normally the quantity of organic iodine lost by either of these routes is probably small and only a few studies of the urinary or fecal excretion of organic iodine in man are available.

3. *Assumptions Concerning the Distribution of a Tracer Dose of Radioactive Iodine:* Radioactive iodine is commonly administered by mouth to subjects in the post-absorptive state. When so given, absorption is quite rapid (182). Hamilton (69) found that absorption was 80 per cent complete within an hour. Keating and Albert (80) state that the rate of absorption is of the order of 5 per cent per minute. At this rate, absorption would be 90 per cent complete in 45 minutes and 99 per cent complete in 90 minutes. These figures indicate that when radioactive iodine is taken by mouth on an empty stomach there will be a brief but appreciable delay before absorption is complete. When given by vein there is of course no delay in absorption, but time will still be required for the radioactive iodine to become evenly distributed throughout the iodide compartment. Brownell (18) estimates that 50 per cent of the final equilibrium concentration is attained in 15 minutes. At this rate 90 per cent of equilibrium would be reached in 50 minutes or 99 per cent in 100 minutes. In the discussion which follows, both the delay in absorption and the delay in distribution will be disregarded, and for the sake of simplicity it will be assumed that the radioactive iodine is instantaneously and evenly distributed throughout the iodide compartment. This assumption is obviously not legitimate if much importance is to be attached to the fate of radioactive iodine during the first hour or two after administration.

Similar assumptions of rapid and uniform distribution throughout the other two compartments will be made. In the thyroid gland the validity of this assumption is somewhat dubious. It is well recognized that in the rat the thyroid follicles lying at the periphery of the gland are less active than the central follicles. Although no such anatomical separation of active and inactive follicles has been described in man, it is quite possible that there may be significant variations in the degree of activity from follicle to follicle. Furthermore, although iodide is swiftly oxidized and combined with tyrosine, conversion to thyroxine requires an appreciable period of time even in the rat where the turnover of organic iodine in the thyroid gland is much more rapid than in man (24). Finally, it might seem reasonable to suppose that newly manufactured hormone would be more available for immediate secretion than hormone previously synthesized and stored as colloid within the lumen of the follicles. Despite these theoretical objections, several studies of the rate of loss of radioactivity from the thyroid gland after the administration of a tracer dose have suggested that for periods of at least several weeks a reasonably constant proportion of the amount remaining in the thyroid gland is lost per unit time (21, 83, 179). This constant rate of loss suggests that the labelled organic iodine is fairly evenly distributed throughout the gland.

The rate of distribution of the thyroid hormone from plasma to tissues is very much slower than the rate of distribution of the iodide ion. However, the bulk of unlabelled hormone already in the tissues is so large compared with the quantity of labelled hormone secreted per day that usually the delay in distribution of radioactive iodine throughout the compartment of extrathyroidal organic iodine can be neglected.

The final proof of the legitimacy of these assumptions and simplifications rests squarely on the ability of the simplified model to explain the observed behavior of iodine in the body and to permit deductions which can later be substantiated experimentally. With further improvements in the techniques of measurement it is quite probable that some of these simplifications will have to be abandoned.

The simplified model of iodine metabolism is depicted diagrammatically in Figure 2. In this and several subsequent figures, the size of each cube representing a compartment is proportional to the quantity of iodine contained by that compartment. The width of each arrow representing a pathway of iodine metabolism is proportional to the quantity of iodine which traverses that pathway per unit time. The model is very similar to the one proposed by Brownell (18), to whom the author owes several of the ideas developed in this paper. It differs from Brownell's model in that the compartment for extrathyroidal organic iodine includes the organic iodine of both plasma and tissues, whereas in Brownell's model the organic iodine in tissues was neglected. It differs substantially from the model proposed by Oddie (126). The inclusion by Oddie of a separate compartment for inorganic iodide within the thyroid gland has already been mentioned. Furthermore, he entirely disregarded the organic iodine present in both plasma and tissue. Since Oddie was chiefly interested in the fate of tracer doses of radioactive iodine during the first 24 to 48 hours after administration, his omission of a compartment for extrathyroidal hormone was perhaps justifiable, at least for euthyroid subjects in whom the rate of turnover of iodine in the thyroid gland is small. Oddie's model will not suffice, however, when the thyroid gland is more active, nor for the interpretation of events occurring over a long period of time.

IV. EQUILIBRIUM STATES AND IODINE BALANCE

Among endocrine organs the thyroid gland is unique in being not only a factory, but also a commodious storehouse for its hormone. This enables it to maintain a steady rate of secretion day after day, despite exceedingly wide fluctuations in the quantity of iodide entering the body. However fortunate this arrangement may be for homeostasis, it seriously hampers the quantitative investigation of thyroid function in man. In the following sections many of the deductions and conclusions will be based on the assumption that the experimental data are obtained from subjects in a state of iodine equilibrium. In other words, it will be assumed that iodine intake precisely balances the loss of iodine from the body, and that the quantity of iodide collected by the thyroid is precisely equal to the quantity of iodine secreted as hormone. In fact, however, the thyroid gland is never in a steady state of equilibrium. Iodide intake is not evenly distributed throughout the 24 hours of the day but is confined almost exclusively

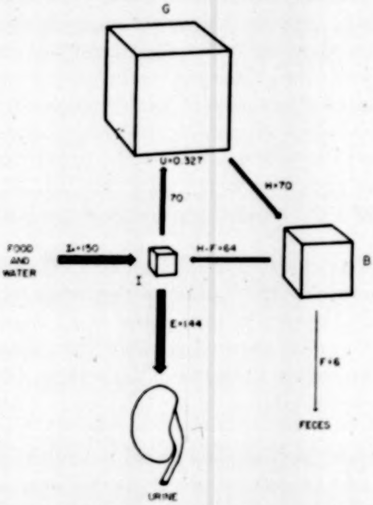

FIG. 2. The simplified model of iodine metabolism upon which is based the discussion in the text. This figure illustrates the metabolism of iodine in a euthyroid subject living in a region where the iodine intake is adequate (Table 3, column 3). The volume of each cube is proportional to the quantity of iodine within the compartment it represents. The width of each arrow is proportional to the micrograms of iodide traversing the designated pathway per day. These rates of transfer are also given by numbers near the arrows. The uptake is given just below the cube representing organic iodine in the thyroid gland. The same mode of construction has been used for Figures 5, 6, 7 and 9. See Section V for explanation of symbols.

to meal-times. Because of the rapidity with which the iodide ion is collected by the thyroid gland and excreted by the kidneys, this irregular intake of iodine causes wide fluctuations from hour to hour in the blood concentration of the iodide ion. Corresponding variations occur in the *absolute quantities* of iodide collected by the thyroid gland and excreted by the kidney, even when the *clearances* remain constant (see Figure 3). Consequently, estimates of the total quantity of iodine handled daily by the body must be based upon analyses of

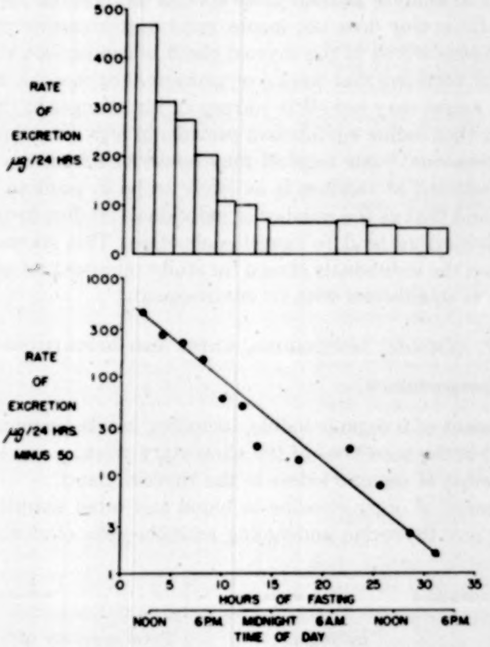

FIG. 3. The renal excretion of iodide during a brief period of starvation. Euthyroid male subject aged 37. The last meal prior to the experiment was a large breakfast, including fish, ending at 9:00 A.M. During the experiment, only distilled water was ingested.

In the upper portion of the figure, the rate of excretion of iodide during successive periods has been plotted as microgm. per day against time. The rate of excretion rapidly approaches a fairly constant value.

In the lower portion of the figure the asymptotic rate of excretion is assumed to be 50 microgm. per day. This value has been subtracted from the observed rates and the differences are plotted on a logarithmic scale against time. The points so obtained can be fitted fairly well by a straight line, thus confirming the assumed value for the asymptote. The 48-hour uptake of radioactive iodine measured at the end of the experiment was 0.401. The mean daily excretion of iodide while the subject was ingesting his usual diet for one day before and six days after the experiment was 148 microgm. H, calculated by equation 15, was 99 microgm./day. F, calculated by equation 28, was 15 microgm./day. In a single experiment of this kind the accuracy with which F can be estimated is low, but it is probably safe to conclude that F is small compared to H.

24-hour urine specimens which have been accurately collected. Furthermore, since considerable variations in the iodine content of the diet occur from day to day, it is best to analyze 24-hour urine specimens collected over a period of a week or more. Even this does not insure valid measurements. So great is the quantity of hormone stored in the thyroid gland in comparison with the normal daily secretion of hormone that weeks, or perhaps even months, of iodine deprivation or slight excess may not elicit corresponding changes in thyroid activity. The assumption that iodine equilibrium prevails in any given individual is evidently a dangerous one. Some comfort may be derived from the statistical fact that a subject selected at random is as likely to be in positive as in negative iodine balance, and that as the number of individuals studied increases, the positive and negative errors tend to cancel each other. This statement obviously applies only when the individuals chosen for study represent an unbiased sample of a population in equilibrium with its environment.

V. SYMBOLS, DIMENSIONS, UNITS AND DEFINITIONS

Designation of compartments.

I. Compartment of inorganic iodide, including iodide present in the thyroid gland and in the secretions of the alimentary tract.
G. Compartment of organic iodine in the thyroid gland.
B. Compartment of organic iodine in blood and other extrathyroidal tissues including any thyroxine undergoing enterohepatic circulation.

SYMBOL	DIMENSIONS	UNITS	DEFINITION
Q_I	m	microgm.	Total quantity of iodide in I
Q_G			Total quantity of iodide in G
Q_B			Total quantity of iodide in B
M_G		gm.	Mass of active thyroid tissue
In	m, t^{-1}	microgm./day	Daily intake of iodide
H			Rate of secretion of hormonal iodine
E			Rate of excretion of iodide by the kidney
F			Rate of excretion of organic iodine (chiefly fecal)
(I)	m, l^{-3}	microgm./l.	Concentration of iodide in I
(B)			Concentration of organic iodine in B
V_I	l^{-3}	liters	Volume of distribution of inorganic iodide in I referred to its concentration in plasma
V_B			Volume of distribution of organic iodine in B referred to its concentration in plasma

SYMBOL	DIMENSIONS	UNITS	DEFINITION
t	t	hours	Time
C_K	l^3, t^{-1}	ml./min.	Renal plasma clearance of iodide
C_G			Thyroid plasma clearance of iodide
ϕ	l^3, m^{-1}, t^{-1}	ml./gm.-min.	Blood flow through the thyroid gland
c		l./microgm.-hr.	Liters of V_B cleared of hormone by breakdown and excretion per microgm. of Q_B per hour
K_{IE}	t^{-1}	proportion per hour	Rate constant of renal excretion of iodide.
K_{IG}			Rate constant of iodide uptake by the thyroid gland.
K_I			Rate constant of loss of iodide from I.
K_{GB}			Rate constant of secretion of organic iodide from thyroid.
K_G			Rate constant of net loss of radioactive iodine from thyroid sufficiently long after a tracer dose so that the ratio of specific activity in G to specific activity in B is constant. Rate of biological decay in G.
K_{BI}			Rate constant of breakdown of hormone in B to inorganic iodide.
K_{BF}			Rate constant of excretion of organic iodine.
K_B			Rate constant of loss of organic iodine from B. Rate of biological decay in B.
U	non-dimensional	proportion	Uptake. Proportion of Q_I accumulated by the thyroid.
P			Extraction efficiency of the thyroid gland. Proportion of iodide in ϕ which is used for synthesis of hormone.
C.R.			Conversion ratio. Proportion of total radioactive iodine in plasma which is organic.

It should be noted that each of the rate constants, K, defined above represents the *proportion* of the total quantity of iodine present in a given compartment which leaves that compartment by a given pathway in unit time. The route and direction of transfer are indicated by an appropriate subscript. Thus K_{GB} is the rate constant of transfer *from* G *to* B. From any such rate constant the *time* required for half of the iodine originally present in the compartment to disappear by the specified route, (the biological half-life), may be calculated by dividing 0.693 (the natural logarithm of 2) by the rate constant.

Symbols used specifically for radioactive iodine will be designated by an asterisk. While quite analogous to the symbols defined above, it must be emphasized that the quantity of radioactive iodine is expressed as a proportion of the administered dose and hence is non-dimensional. In order to avoid difficulty with dimensions, therefore, the symbols employed for radioactive iodine are listed below:

SYMBOL	DIMENSIONS	UNITS	DEFINITION
Q_I^* etc.	non-dimensional	proportion	Proportion of the dose in I etc.
Q_E^*			Proportion of the dose which has been excreted as iodide.
Q_F^*			Proportion of the dose which has been excreted as organic iodine.
H^*, E^*, F^*	t^{-1}	proportion per day	Proportion of the dose secreted as hormone per day etc.
$(I)^*$, $(B)^*$	l^{-3}	proportion per liter	Concentration of radioactive iodine in I etc.

Subscripts are sometimes used to modify the symbols defined above. The meanings of these subscripts are listed below:

0	at time zero.
t	at time t.
A	in arterial blood.
TV	in thyroid vein blood.
s	during starvation.
bl	during a block of the synthesis of thyroid hormone.

The reviewer is quite aware of the confusion which may arise from using different units for the same dimension. However, it would undoubtedly be even more confusing to express the intake of iodide in gm. per hr. or the renal clearance of iodide in liters per hr. For the most part, therefore, it has seemed wise to express quantities in the units which are traditional and familiar, and to employ unit-conversion factors where necessary in the equations. However, the concentrations of iodine have been expressed as microgm. per liter rather than microgm. per cent, and all non-dimensional quantities have been expressed as proportion rather than as per cent. Since these departures from custom are in the decimal system, they should cause no difficulty.

VI. FACTORS WHICH INFLUENCE THE ACTIVITY OF THE THYROID GLAND

The activity of the normal thyroid gland is regulated chiefly, if not wholly, by the thyrotropic hormone of the anterior pituitary (58, 117, 129, 138). If thyroid activity becomes insufficient to maintain a normal concentration of thyroid hormone in the blood stream, the anterior pituitary, by increasing its secretion of thyrotropic hormone, stimulates the thyroid gland to greater activity. Conversely, if the thyroid becomes too active, the increased concentration of circulating thyroid hormone causes inhibition of the secretion of thyrotropic hormone with a compensatory decrease in thyroid function.

The most obvious effect of thyrotropic hormone on the thyroid gland is to cause hypertrophy and hyperplasia which increase the mass of active tissue, M_G. Concomitantly the blood supply also increases. The increase in vascularity may be out of proportion to the increase in mass of active tissue so that the blood flow per gram of tissue, ϕ, increases. Finally the efficiency, P, with which the gland is able to extract iodide from the blood stream and convert it to organic iodine (184) may also be increased. Therefore any factor, alteration of which demands a change in M_G, ϕ or P for maintenance of equilibrium, is a factor which influences thyroid activity. These factors may be expressed in a single equation derived from two separate equations for the rate at which the thyroid clears iodide from the plasma.

The clearance of iodide from *whole blood* by the thyroid gland is equal to the product of the total thyroid blood flow per minute, $M_G\phi$, and the efficiency of extraction P. To convert whole blood clearance, $M_G\ \phi\ P$, to plasma clearance, C_G, it is necessary to correct for the iodide in erythrocytes, which presumably is also available to the thyroid gland (see Section III, A). Taking the water content of plasma as 0.92 and of red blood cells as 0.60, and assuming a normal hematocrit of 0.45 (15), the concentration of iodide in plasma will be about 1.19 times the concentration in whole blood. Hence the *plasma* iodide clearance will be $\frac{1}{1.19}$ times the whole blood iodide clearance:

$$C_G = \frac{M_G\ \phi\ P}{1.19} \qquad 1$$

The plasma clearance of iodide by the thyroid will also be equal to the amount of iodide converted to organic iodine per minute divided by the concentration of iodide per ml. of plasma, (I)/1000. But at equilibrium the amount of iodide organically bound by the thyroid per minute will equal the amount secreted as hormone per minute, H/1440. Hence:

$$C_G = \frac{H}{1.44\ (I)} \qquad 2$$

At equilibrium also, the intake of iodine will equal the total excretion of iodine:—

$$In = E + F \qquad 3$$

or

$$E = In - F \qquad 3a$$

Furthermore, the concentration of iodide per liter of plasma may be calculated from the quantity excreted per minute, E/1440, and the plasma clearance of iodide by the kidney[2], C_K,:

$$C_K = \frac{E}{1.44\ (I)} \qquad 4$$

Substituting the value of E from equation 3a in equation 4, and solving for (I):

$$C_K = \frac{In - F}{1.44\ (I)} \qquad 5$$

$$(I) = \frac{In - F}{1.44 C_K} \qquad 5a$$

Substituting this value for (I) in equation 2:

$$C_G = \frac{C_K H}{In - F} \qquad 6$$

But equations 6 and 1 are both expressions for the plasma clearance of iodide by the thyroid gland, and hence may be equated to each other:

$$\frac{M_G \phi P}{1.19} = \frac{C_K H}{In - F} \qquad 7$$

Equation 7 could be solved for either M_G, ϕ or P. However, the extent to which ϕ and P can increase with increasing thyroid activity is limited. Furthermore P can be profoundly altered by pharmacological agents (see Section X). In contrast, M_G can increase with thyroid activity almost without limit (50) and is probably not directly influenced by drugs other than thyrotropic hormone. It therefore seems logical to solve equation 7 for M_G:

$$M_G = \frac{1.19\ C_K H}{\phi P(In - F)} \qquad 8$$

Equation 8 states that at equilibrium the *mass of active thyroid tissue* is directly proportional to the *renal clearance* of iodide and the *rate of secretion* of the thyroid hormone, and inversely proportional to the *blood flow per gram of tissue*, the *efficiency of extraction* and the difference between *iodide intake* and *excretion of organic iodine*. Together, these seven factors completely determine the activity of the thyroid gland. In subsequent sections each of these factors will be considered in turn.

Equation 8 should not be interpreted to mean that a change in one of the other factors *necessarily* causes a change in the mass of active thyroid tissue. For example, equilibrium could be maintained in the face of an increased intake of iodide by appropriate decreases in blood flow or extraction efficiency, or both, without any change in M_G.

---

[2] Since iodide appears to be excreted by glomerular filtration, and reabsorption (see Section VI, A), the iodide in erythrocytes is not immediately available for excretion during a single passage of blood through the kidney, and no correction of the renal plasma clearance for iodide in erythrocytes need be made.

A. *The Renal Clearance of Iodide:* The rate at which iodide is cleared from the plasma by the kidneys may be calculated in the usual manner as the quantity of iodide excreted per minute divided by the concentration of iodide per ml. of plasma (equation 4). If the clearance is to be calculated from chemical determinations of iodide in urine and plasma, the concentration must be deliberately increased by the administration of iodide, since the concentration of inorganic iodide normally present in the plasma is far too small for accurate chemical analysis. This disadvantage is avoided by the administration of carrier-free radioactive iodine which will not appreciably alter the total quantity of iodide within the body. During the first few hours after the administration of a tracer dose, it is usually safe to assume that all of the radioactivity in the blood stream is inorganic iodide, and simple measurements of the total quantity of radioactivity in the plasma and in the urine will suffice for estimation of the renal clearance. However, it is preferable to remove the organic iodine from samples of plasma or serum by precipitating the protein and to measure the radioactive iodide which remains in the supernatant fluid. This precaution becomes essential when the turnover of iodine in the thyroid gland is unusually rapid.

Smith has discussed the theoretical objections to calculation of clearances after the administration of a single dose (176). He believes that reliable results cannot be obtained even when due allowance is made for the logarithmic decrease in blood concentration during the collection periods, and suggests that accurate estimations of renal clearance can be obtained only when the blood concentration is maintained at a constant level by continuous intravenous infusion. Unfortunately the reviewer is unaware of any studies in man during which the blood concentration of either I^{127} or I^{131} was maintained by continuous intravenous infusion. Despite Smith's cogent objections, estimates of the renal clearance of iodide using the single dose technique are probably accurate enough for most purposes.

When serial measurements of radioactive iodine in samples of plasma and urine are available, Berkson and his collaborators (13) consider it more accurate to calculate the renal clearance from the rate of disappearance of radioactivity from the plasma, $K_{IE} + K_{IG}$, the plasma concentration at zero time, $(I)_0^*$, and the proportion of the tracer dose excreted in the urine $1 - U$. The reader is referred to the original paper for a full discussion of the assumptions involved, and for a detailed description of the mode of calculation. In brief, the equation for this method may be derived as follows: By definition, the renal clearance is the proportion of the volume of distribution of iodide which is cleared per minute, multiplied by the volume of distribution. Hence:

$$C_K = \frac{K_{IE}}{60}(1000\ V_I) = \frac{100\ K_{IE} V_I}{6} \qquad 9$$

But K_{IE}, the rate constant of excretion, is equal to the proportion of the dose excreted in the urine, multiplied by the total rate of disappearance from the plasma:

$$K_{IE} = (1 - U)\,(K_{IE} + K_{IG}) \tag{10}$$

And since at zero time the entire dose is assumed to be evenly distributed throughout the volume of distribution:

TABLE 1

The renal clearance of radioactive iodide

SUBJECTS	NUMBER OF SUBJECTS	RENAL CLEARANCE OF I^{131} ML. OF PLASMA PER MINUTE			REFERENCE NUMBER
		Minimum	Maximum	Mean*	
Euthyroid	7	11	44	31	123
Euthyroid (Medical Students)	12	21	53	36	119
Euthyroid	9			33.3 ± 3.1	105
Euthyroid weighted mean	28			34	
Euthyroid Renal Function Impaired	12			8.7 ± 1.6	105
Hyperthyroid	5				28
I^{127} carrier					
0.001 mgm.		40	49	44.3 ± 2.0	
0.1 mgm.		26	53	36.9 ± 4.5	
1.0 mgm.		29	52	34.3 ± 5.6	
10.0 mgm.		25	52	37.1 ± 4.9	
Hyperthyroid	16			32.7 ± 1.0	105
Hyperthyroid	11	10	46	27	123
Hyperthyroid weighted mean	32			32	
Myxedema	5			17.9 ± 1.6	105

* When available, the standard error of the mean is also given.

$$V_I = \frac{1}{(I)_0^*} \tag{11}$$

Substituting the values from equations 10 and 11 in equation 9,

$$C_K = \frac{100\,(1 - U)(K_{IE} + K_{IG})}{6(I)_0^*} \tag{12}$$

Berkson and his collaborators have shown that the agreement between these two methods of calculation is usually very satisfactory. In a series of 40 patients the mean clearance determined by the classical method was 31.7 ml. of plasma per minute. The mean clearance by the method just described was 30.7 ml. of plasma per minute.

Table 1 summarizes values for the renal clearance of iodide from the plasma under various circumstances. The mean clearance among euthyroid subjects is about 34 ml. of plasma per minute with considerable variation from individual to individual. For convenience in calculation, the mean normal value will henceforth be taken as $33\frac{1}{3}$ ml. of plasma per minute. It is also evident from Table 1 that no significant change in the renal iodide clearance occurs during hyperthyroidism. However, in myxedema the renal clearance of iodide may be significantly decreased, and in patients with renal disease the ability of the kidneys to clear iodide from the blood stream is often greatly impaired.

The clearance of iodide by the human kidney is very much greater than the clearances of chloride, bromide or thiocyanate, but is still only about one quarter of the glomerular filtration rate. Presumably this means that approximately three quarters of the iodide filtered by the glomeruli is reabsorbed by the renal tubules.

The renal clearance of iodide is remarkably constant over an enormous range of plasma concentrations. When the plasma concentration is raised to 100,000 microgm. per liter by the administration of carrier iodide, the renal clearance of radioactive iodine is approximately the same as when the radioactive iodine is given without carrier (28). Furthermore, no reduction in renal clearance occurs in regions of iodine deficiency where the mean concentration of iodide ion in the plasma may be as low as 0.2 microgm. per liter (179). This complete indifference of the kidney to the need for conservation of iodide imposes a heavy burden upon the thyroid gland when the supply of iodine is restricted.

In man the renal excretion of the iodide ion, unlike that of the bromide ion, is but little influenced by extreme variations in the quantity of chloride being excreted simultaneously (159). In contrast, in the dog the renal clearance of iodide is largely determined by the rate of chloride excretion. The clearance of iodide in this species may rapidly change from less than 0.1 ml. of plasma per minute when chloride intake is restricted, to over 30 ml. of plasma per minute when large quantities of chloride are administered (156).

Because of the keen competition between the thyroid gland and the kidneys for the available supply of iodide, impairment of renal function severe enough to reduce the renal iodide clearance makes it much easier for the thyroid gland to obtain its daily allotment of iodide. Renal impairment should therefore provide partial protection from thyroid enlargement due to iodine deficiency since a decrease in C_K will tend to counterbalance a decrease of In (see equation 8).

B. *The Rate of Secretion of the Thyroid Hormone:* The rate of secretion of the thyroid hormone cannot be directly measured. Even if it were technically possible to obtain pure samples of venous blood from the thyroid gland in man, the arterio-venous difference in concentration of thyroid hormone would be far too small to be detectable by present analytical methods. If the plasma entering the thyroid gland contained 50 microgm. of protein-bound iodine per liter and if the rate of secretion of thyroid hormone were normal, the plasma leaving the gland would contain about 51 microgm. per liter, an increase of only 2 per cent. Reliance must therefore be placed on indirect methods for estimating the rate of hormone secretion.

1. *Methods Based on Substitution Therapy:* In the past it has been customary

to consider the quantity of exogenous hormone which must be supplied daily to a patient with complete myxedema as equal to the normal quantity of hormone secreted daily by the thyroid gland. This involves the assumption that the exogenous hormone is utilized as efficiently as endogenous hormone. Plummer and Boothby (136) estimated that approximately 300 microgm. of racemic thyroxine are required daily to maintain patients with severe myxedema in a euthyroid state. In a very thorough study of two patients with myxedema Thompson and his collaborators found that the daily requirement was about 325 microgm. of racemic thyroxine (191). Since d-thyroxine is only one tenth as potent as l-thyroxine (134), 325 microgm. of the racemic drug is equivalent to 179 microgm. of the naturally-occurring levorotatory isomer, or to 116 micrograms of hormonal iodine per day.

Somewhat smaller amounts of hormone appear to suffice when desiccated thyroid is administered by mouth. From his very wide experience, Means estimates the daily requirement as 100 mgm. of U.S.P. thyroid (107). Winkler and his collaborators (201) found that an increase of serum iodine averaging 2.0 microgm. per cent occurred for each 65 mgm. of thyroid administered to patients with hypothyroidism. It would therefore require approximately 160 mgm. of thyroid daily to increase the concentration of iodine in the serum from zero to a normal level of 5 microgm. per cent. Among 25 normal subjects studied by Greer (59), the daily amount of thyroid needed to decrease the uptake of radioactive iodine to less than 10 per cent of the administered dose was 65 mgm. in 9 subjects, 130 mgm. in 12 subjects, 195 mgm. in 2 subjects, and 260 mgm. in 2 subjects. The mean quantity required for marked (but not necessarily complete) suppression of thyroid activity was therefore approximately 120 mgm. per day. As a compromise between these three estimates, 130 mgm. may be taken as the mean quantity of thyroid needed per day for complete substitution therapy in patients without functional thyroid tissue. Although the total quantity of organic iodine in 130 mgm. of U.S.P. thyroid is 260 ± 39 microgm., only about one third of this, or 87 microgm., is present as thyroxine. The remainder is present as iodinated tyrosine, which is physiologically inert.

The supposed discrepancy between the quantity of thyroxine required when given as the pure substance by vein and when given by mouth in the form of desiccated thyroid has long been a matter of comment. The statement is often made that the potency of dried thyroid is greater than can be accounted for by its thyroxine content, the implication being that in some mysterious way the other iodine-containing compounds become endowed with calorigenic activity (93, 111). But when due allowance is made for the relative activity of the optical isomers of thyroxine, the discrepancy between the potency of thyroid by mouth and thyroxine by vein almost disappears. Furthermore, recent investigations of the fate of thyroxine in animals and in humans provide a reasonable explanation for whatever small discrepancy remains. Gross and Leblond (62) have shown that in the rat over two thirds of an unphysiologically large dose of radioactive thyroxine is excreted in the feces during 24 hours. When physiological doses are used, much smaller amounts are excreted. In man, thyroxine is usually given by vein and, since the rate at which it leaves the blood stream is rather slow

(2, 122, 153), abnormally high concentrations which may favor excretion by the liver are undoubtedly present in the plasma for several hours after an intravenous injection.

An ingenious experiment by Myant and Pochin (122) casts further doubt on the equivalence of injected thyroxine and endogenous thyroid hormone. In three euthyroid subjects the behavior of synthetic dl-thyroxine labelled with radioactive iodine was compared with radioactive hormone synthesized by the thyroid glands of three thyrotoxic patients who had received large therapeutic doses of radioactive iodine. Three days after administration of the therapeutic doses 20 ml. aliquots of plasma from the thyrotoxic patients were injected into the normal subjects. The "natural thyroxine" disappeared from the blood stream at a much slower rate than synthetic radioactive thyroxine. Twenty-four hours after injection, the mean concentration of natural thyroxine was 14.8 per cent of the dose per liter of plasma, while the mean concentration of synthetic thyroxine was only 3.6 per cent of the dose per liter of plasma. Since the dose of synthetic thyroxine was only 80 microgm., the blood concentration, even immediately after injection, was probably not high enough to favor increased excretion by the liver. The observed difference in rate of disappearance cannot be ascribed to the use of racemic thyroxine, since in two control experiments the distribution of pure synthetic l-thyroxine was similar to the distribution of the racemic compound. By demonstrating that the majority of the organically bound radioactivity could be extracted with butanol in which thyroglobulin is not soluble, the authors also excluded the possibility that the samples of serum from the thyrotoxic patients might have contained thyroglobulin rather than thyroxine. They concluded that thyroglobulin could not have constituted more than a small fraction of the radioactive iodine in the plasma. The authors suggest that the linkage between injected thyroxine and the plasma proteins may differ from the linkage which occurs when thyroxine is secreted from the thyroid gland into the blood stream. This explanation is also supported by the experiments of Salter and Johnson (168). However, Gordon and his collaborators (53) have recently demonstrated that when serum from patients treated with large doses of radioactive iodine is subjected to electrophoresis on filter paper, practically all of the organic iodine accompanies α_1 globulin. Thyroxine mixed with plasma *in vitro* behaved in precisely the same fashion. This constitutes strong presumptive evidence that the binding of exogenous thyroxine is similar to the binding of endogenous hormone.

While the evidence is conflicting, it is likely that injected thyroxine is not utilized quite as efficiently as hormone released from the thyroid gland. When desiccated thyroid is given by mouth, the absorption of hormone from the gastro-intestinal tract may be slow enough to permit more efficient utilization. It seems highly probable that the calorigenic activity of desiccated thyroid is fully accounted for by its thyroxine content. However, it is unlikely that absorption and utilization is 100 per cent efficient. Consequently, the 85 to 90 microgm. of l-thyroxine iodine required daily in this form is probably somewhat greater than the daily secretion of hormonal iodine from the normal thyroid gland.

2. *Methods Based on the Uptake of Radioactive Iodine by the Gland and the*

Excretion of Stable Iodine in the Urine: Since the thyroid gland does not distinguish between I^{131} and I^{127}, the proportion of a tracer dose of radioactive iodine which is accumulated by the thyroid is also the proportion of *all* of the iodide in the body which is accumulated. If equilibrium is maintained, it is likewise the proportion of all of the iodide *entering* the iodide compartment which is accumulated. The iodide entering is the sum of In, the iodide absorbed from the gastro-intestinal tract, and H − F, the iodide returned to the iodide compartment by breakdown of thyroid hormone in the tissues. The total quantity of iodide entering the thyroid gland in 24 hours is therefore U(In + H − F). But at equilibrium the quantity of iodide entering the gland is equal to the quantity secreted as hormone. Hence:

$$\mathrm{H} = \mathrm{U}(\mathrm{In} + \mathrm{H} - \mathrm{F}) \qquad 13$$

From equation 3:

$$\mathrm{In} = \mathrm{E} + \mathrm{F} \qquad 3$$

substituting this value in equation 13:

$$\mathrm{H} = \mathrm{U}(\mathrm{E} + \mathrm{H}) \qquad 14$$

Solving for H:

$$\mathrm{H} = \frac{\mathrm{UE}}{1 - \mathrm{U}} \qquad 15$$

The same equation can be derived more simply, but perhaps less instructively, from the fact that the ratio of iodide collected to iodide excreted is $\frac{\mathrm{U}}{1 - \mathrm{U}}$. Hence, at equilibrium:

$$\frac{\mathrm{H}}{\mathrm{E}} = \frac{\mathrm{U}}{1 - \mathrm{U}} \qquad 16$$

The dangers inherent in the assumption of equilibrium have already been discussed. Consequently, equation 15 is most useful when H can be calculated from studies of a considerable number of euthyroid individuals. For this purpose a logarithmic transformation is particularly useful. Solving equation 15 for E,

$$\mathrm{E} = \frac{\mathrm{H}(1 - \mathrm{U})}{\mathrm{U}} = \mathrm{H}\left(\frac{1}{\mathrm{U}} - 1\right) \qquad 17$$

$$\log \mathrm{E} = \log \mathrm{H} + \log\left(\frac{1}{\mathrm{U}} - 1\right) \qquad 18$$

It is evident from equation 18 that a plot of log E against $\log\left(\frac{1}{\mathrm{U}} - 1\right)$ should give a straight line with a slope of unity and that the intercept of this line with the zero axis for $\log\left(\frac{1}{\mathrm{U}} - 1\right)$ should provide an estimate of log H. In practice the uptake of radioactive iodine and the excretion of I^{127} in 24-hour urine specimens are measured in a number of euthyroid individuals, and the observed values for E and U are appropriately transformed and plotted according to equation 18. The best straight line with a slope of unity is fitted to the points and log H is read as the value of log E when $\log\left(\frac{1}{U} - 1\right)$ is zero (*i.e.*, when $U = 0.5$).

This mode of calculation has been used in the analysis of data obtained from euthyroid subjects in a region of iodine deficiency (179). A crude estimate of 45 microgm. per day was obtained for the normal rate of secretion of hormonal iodine. The data are subject to a number of corrections which will undoubtedly increase the calculated value. Moreover, because of the difficulty of persuading a large number of normal subjects to collect accurate and complete 24-hour urine specimens, this method is almost certain to underestimate the true value for the rate of secretion of the thyroid hormone.

If the rate of excretion of organic iodine is known, or if it is small enough to be negligible, the rate of secretion of hormone from the thyroid gland may also be calculated from the excretion of I^{127} in fasting subjects. This method will be discussed below in the section on the excretion of organic iodine.

3. *Methods Based on Specific Activity:* Stanley has pointed out that the quantity of I^{127} accumulated by the thyroid gland can be calculated from the excretion of I^{127} in the urine and the specific activity of the urine (181). Since the thyroid and the kidneys do not distinguish between stable and radioactive iodine, at any given moment the iodide being accumulated by the thyroid gland or being excreted in the urine must have the same specific activity as the iodide in the blood stream. Hence:

$$\frac{I^{131}\text{ entering thyroid}_t}{I^{127}\text{ entering thyroid}_t} = \frac{I^{131}\text{ in urine}_t}{I^{127}\text{ in urine}_t} = \frac{(I)^{*}_t}{(I)_t} \qquad 19$$

and,

$$I^{127}\text{ entering thyroid}_t = \frac{(I^{127}\text{ in urine})_t\,(I^{131}\text{ entering thyroid})_t}{(I^{131}\text{ in urine})_t} \qquad 20$$

After a single dose of radioactive iodine it is obvious that the blood concentration of I^{131} will fall rapidly as the isotope is trapped by the thyroid gland or excreted in the urine. The concentration of I^{127}, on the other hand, will be influenced not only by the activity of the thyroid and the kidneys but also by the quantity of I^{127} being added to the iodide compartment continually by breakdown of hormone in the tissues, and intermittently by absorption from the gastro-intestinal tract. Consequently, the specific activity will decrease rather rapidly with the passage of time. This will not affect the validity of equations 19 and 20, since the change in specific activity will be the same for each of the three components of the equation. Since equation 20 is valid for each instant of time, it is also valid for the total amount collected and excreted during any given *interval* of time, provided that C_K and C_G remain constant. If enough time is allowed for all of the radioactive iodine to be excreted or accumulated, the method of equation 20 reduces to that of equation 15. However, equation 20 is somewhat more versatile than equation 15. Since it does not require the assumption of equilibrium, it can be used for the calculation of the quantity of stable iodide entering the thyroid gland during brief periods of time. It thus facilitates the investigation of factors which alter the quantity of iodide accumulated. Using this method, Stanley has been able to demonstrate the transient influence of iodide in food or medication on the quantity of iodide entering the thyroid. It should be evident from the previous discussion, however, that reliable estimates of the rate of *secretion* of hormone cannot be obtained when equation 20 is applied to observations made during a short period of time. For example, Stanley reported that the rate of accumulation of stable iodide in 14 euthyroid subjects who had not received additional iodide varied from 3 to 19 microgm. per hour, averaging 10 microgm. per hour, for periods of from 6 to 28 hours. If the mean rate of 10 microgm. per hour were assumed to be representative of iodide accumulation over a long period of time, it would imply that the normal rate of secretion was approximately 240 microgm. of hormonal iodine per day. This estimate is considerably higher than the ones discussed previously and probably indicates that during the period of study most of Stanley's subjects were accumulating more than their equilibrium allotment of iodide.

Theoretically it should be possible to calculate the rate of secretion of hormonal iodine from the thyroid gland by measuring the increase in the excretion of stable iodide which occurs when the synthesis of hormone is blocked by the administration of a drug such as 1-methyl-2-mercaptoimidazol. For this method of calculation, it is necessary to assume that the sole action of the drug is to inhibit the uptake of iodide by the thyroid gland, and that loss of iodine from the gland and return of iodide from the tissues remain constant throughout the period of study. It is also necessary to determine the uptake of radioactive iodine by the blocked gland as a measure of the degree of block produced. During the block the quantity of iodide excreted per day in the urine will be equal to the proportion of the tracer dose excreted multiplied by the quantity of iodide entering the iodide compartment:

$$E_{bl} = (1 - U_{bl})\,(In + H - F) \qquad 21$$

Substituting in equation 21 the value for In − F from equation 3a, and solving for H:

$$E_{bl} = (1 - U_{bl})(E + H)$$

$$H = \frac{E_{bl}}{1 - U_{bl}} - E \qquad 22$$

If the block is complete, U_{bl} becomes equal to 0, and the equation reduces to:

$$H = E_{bl} - E \qquad 23$$

Unexpected difficulties were encountered when this mode of calculation was applied (179). The difference between the excretion of stable iodide before and during the administration of l-methyl-2-mercaptoimidazol was much larger than the daily secretion of hormone estimated by previous methods. Furthermore, it is possible to predict from U_{b1} and U the ratio to be expected between E_{b1} and E. Substituting in equation 22 the value for H from equation 15

$$\frac{UE}{1 - U} = \frac{E_{b1}}{1 - U_{b1}} - E \qquad 24$$

Solving equation 24 for $\frac{E_{b1}}{E}$:

$$E\left(\frac{U}{1 - U} + 1\right) = \frac{E}{1 - U} = \frac{E_{b1}}{1 - U_{b1}}$$

$$\frac{E_{b1}}{E} = \frac{1 - U_{b1}}{1 - U} \qquad 25$$

The observed ratio of E_{b1} to E was considerably greater than the ratio predicted by equation 25. This calls into serious question the validity of the basic assumption that the blocking agent did not affect the rate of loss of iodine from the thyroid gland. It seems possible that blocking agents of this type increase the rate at which iodine leaves the gland. Albert and Rawson have suggested that they may potentiate the action of thyrotropic hormone (4). If so, the extra iodine lost from the thyroid would presumably be secreted as thyroxine. This should cause a *gradual* increase in the excretion of iodide. But in the study described above, the increased excretion of iodide occurred rather promptly. It seems more probable that the extra iodine leaves the gland either as iodinated tyrosine or as iodide derived from the breakdown of iodinated tyrosine. This peculiar phenomenon deserves further investigation.

4. *Other Methods:* Salter presents several equations for the calculation of what he terms the "thyroxine metabolic turnover rate" or "TMR" which he defines as the daily production of l-thyroxine expressed as micrograms of organic iodine (164, 166, 167). Unfortunately, he gives no satisfactory derivation for these equations and does not even define some of the symbols employed. Furthermore, it is easy to show by dimensional analysis that two of his equations cannot possibly be equivalent mathematically.[3] It would therefore seem pointless to discuss them further.

---

[3] Salter states (167):—"Theoretically,

$$\text{TMR} = \frac{\text{Plasma volume}}{100} \times \text{SHI} \ln \text{SHI} \times \text{K} = \text{K}\,\frac{\text{serum radioactivity}}{\text{per 100 ml.}} \times \left(\frac{\text{plasma volume}}{100}\right)^2$$

Where
TMR = daily production of l-thyroxine (expressed as micrograms of organic iodine);
SHI = serum protein-bound iodine in micrograms per 100 ml.; and
K = the labelling constant which is close to 0.4 in value."

If we assume that by $\frac{\text{“serum radioactivity}}{\text{per 100 ml.”}}$ is simply meant "serum radioactivity per 100 ml." the second and third portions of this "equation" may be reduced to:—

While it is not possible at the present time to give a final value for the normal rate of secretion of the thyroid hormone in man, we are fortunate in having one estimate (based upon replacement therapy) which is probably too high, and another estimate (based upon the excretion of stable iodine in the urine and the uptake of radioactive iodine) which is certainly too low. The true value must lie somewhere between these two independent estimates. Pending more precise measurements, we may accept a value of 70 microgm. of thyroxine iodine per day as the mean rate of secretion in the normal human. This figure is certainly of the correct order of magnitude and is probably rather close to the true value.

C. *The Daily Intake of Iodine:* There is no such thing as a "normal" intake of iodine. The ability of the thyroid gland to compensate for changes in iodine supply is so great that it can maintain a normal rate of secretion whether the intake be 10 microgm. per day, as in regions of severe iodine deficiency, or several million microgm. per day, as when large doses of iodide are administered in the treatment of syphilis and fungus infections. The reviewer will adopt the chauvinistic attitude that the iodine intake in Boston and similar seacoast regions is normal. Even in such regions of natural iodine abundance, great variation must be anticipated because of dietary vagaries, the deliberate or unwitting use of iodized salt, the employment of iodate in certain bread conditioners, and the countless ointments, toothpastes and drugs which contain relatively enormous quantities of iodine.

In persons eating a normal mixed diet it is practically impossible to obtain reliable measurements of iodine intake by the chemical analysis of food and water. The formidable difficulties of sampling foodstuffs without bias, and the decreased reliability of analytical results when large amounts of fat are present in the material to be analyzed, have discouraged recent investigators from attempting the direct measurement of iodine intake. In the next section it will be shown that the loss of organic iodine from the body in both urine and feces is probably small. Reasonably reliable estimates of intake may therefore be obtained by chemical analysis of 24-hour urine specimens. In Table 2 are summarized the results of several studies of the daily excretion of iodide in regions without endemic goiter. Most of the values were obtained from the excellent monograph by McClendon (101). The mean is close to 150 microgm. per day. The mean intake of iodine in food and water is therefore slightly greater than this. For ease in calculation, 144 microgm. per day will be taken as the "normal" daily excretion of iodide in the urine.

The effects on thyroid function of iodine deficiency (see Section VIII, F) and of iodine excess (see Section X, B) will be discussed later.

D. *The Excretion of Organic Iodine:* In the previous discussion it has been convenient to represent the organic iodine lost from the body as a single entity.

SHI ln SHI = serum radioactivity per 100 ml. $\left(\frac{\text{plasma volume}}{100}\right)$

The dimensional "equation" for this is:—

$m, l^{-3} = l^{-3}, l^{3}$

which is impossible.

It is now necessary to consider urinary and fecal losses separately since there is evidence that small quantities of organic iodine may be excreted by both of these routes.

1. *Urine:* Before the advent of radioactive iodine, attempts to demonstrate organic compounds of iodine in the urine of normal or hyperthyroid subjects by chemical methods had repeatedly failed, and it was generally conceded that most, if not all, of the urinary iodine was inorganic (40, 43). This comfortable belief has recently been challenged by Rall (142) who has studied the excretion of radioactive iodine in urine samples fractionated by chemical and physical methods. In the urine of normal individuals only minute traces of iodine precipitable with zinc hydroxide were found 72 hours after a tracer dose. At this time practically all of the radioactive iodide originally administered had presumably either been excreted by the kidneys or accumulated by the thyroid gland. It seems probable, therefore, that the radioactivity appearing at 72 hours was derived chiefly from labelled thyroid hormone which had been synthesized and secreted by the thyroid gland and metabolized by the tissues. This suggests that in normal subjects the urinary excretion of organic iodine is negligible. In hyperthyroid subjects, however, approximately 25 per cent of the radioactive iodine in the urine 72 hours after a tracer dose was precipitable with zinc. Since in hyperthyroidism the rate of accumulation of iodide in the thyroid gland is so rapid, it is almost certain that the radioactivity in the urine represented only iodine derived from labelled hormone. Indeed, Rall (142) points out that the appearance of precipitable radioactive iodine in the urine closely parallels the appearance of precipitable radioactivity in the serum (104). Since Rall did not report the uptake of radioactive iodine in these subjects, it is difficult to calcu-

TABLE 2

The urinary excretion of iodine in regions free of endemic goiter

INVESTIGATOR	LOCALITY	IODINE IN 24-HOUR SAMPLE	REFERENCE
		microgm.	
Von Fellenberg	Vik i Sogn, Norway	146	McClendon (101) page 53
Lunde	Vik i Sogn, Norway	173	McClendon (101) Table 53
Reith	Barendrecht, Holland	186	McClendon (101) Table 54
Kupzis	Latvia	(81 to 157)	McClendon (101) Table 54
Hercus and Roberts	New Zealand	49	McClendon (101) Table 54
Triebart	Batavia	126	McClendon (101) Table 54
Triebart	Java	135	McClendon (101) Table 54
Bruger	New York, N. Y.	191	Salter (162) page 79
Stanbury *et al.*	Boston	125	Unpublished data
Liek	Danzig	343	Curtis *et al.* (35) Table 2
Scheringer	Berlin	141	Curtis *et al.* (35) Table 2
Moore	New Orleans	117	Curtis *et al.* (35) Table 2
Von Fellenberg	Forte dei Marmi, Italy	72	Curtis *et al.* (35) Table 2
Mean (omitting value in parenthesis)		150.3	

late the proportion of the organic iodine secreted by the thyroid gland which was being lost from the body in the urine. However, if it be assumed that the mean uptake was 75 per cent, the following calculation will provide a rough estimate of the proportion of hormone lost in the urine as organic iodine:

Let X equal the total amount of I^{131} in the urine at 72 hours.

Then 0.75X equals the inorganic I^{131} in the urine.

But this represents only 0.25 of the total amount of I^{131} returned to the blood stream by breakdown of labelled hormone in the tissues.

Hence, total amount of I^{131} returned to the iodide compartment $= \frac{0.75\,X}{0.25} = 3X$.

Organic I^{131} lost in urine $= 0.25\,X$.

Total labelled hormone turnover $= 3.25X$.

Proportion lost in urine as organic $I^{131} = \frac{0.25X}{3.25X} = 0.077$.

In this calculation, fecal excretion has been neglected, and it has been assumed that at 72 hours the proportion of the radioactivity in the urine which is organic is the equilibrium proportion. Actually Rall's figures suggest that the proportion may still be increasing slowly at 72 hours. However, insofar as this calculation is valid it suggests that even in hyperthyroidism less than 10 per cent of the hormone secreted by the thyroid appears in the urine as organic iodine.

If the values for thyrotoxicosis listed in Table 3 be taken as representative, the turnover of hormone per day (neglecting fecal loss) would be 597 microgm. According to the calculation above, 46 microgm. of this might be excreted in organic form. In addition, 199 microgm. of inorganic iodide would be excreted. Of the total iodine in the urine $\frac{46}{245}$ or 0.19 would be organic. If as much as 19 per cent of the iodine being excreted in hyperthyroidism is organic iodine, it is rather surprising that it has not been detected by chemical methods. The problem should certainly be re-examined with the improved chemical techniques which are now available.

Rall also separated the organic iodine in the urine into thyroxine-like and diiodotyrosine-like fractions. Unfortunately, the tabulation of results which he presents does not indicate at what interval after the tracer the urines were collected. In one patient with myxedema, three euthyroid subjects, and six patients with hyperthyroidism, only 2 per cent or less of the radioactive iodine in the urine was found in the thyroxine fraction. However, in four other patients with hyperthyroidism from 3 to 10 per cent behaved like thyroxine. By adding carrier thyroxine to butanol extracts of urine and recrystallizing to constant specific activity, and by chromatographic separation on filter paper, Rall appears to have conclusively demonstrated the presence of at least some thyroxine in the urine.

The proportion of the radioactive iodine in the urine which was recovered in the diiodotyrosine fraction was extremely variable, ranging from 1.4 per cent in a patient with myxedema, to as much as 68 per cent in a patient with exophthalmic goiter. In the 10 patients with hyperthyroidism the mean proportion was 28 per cent. It is difficult to reconcile these observations with a later study by Albert and Keating (3) who clearly showed that when radioactive diiodotyrosine was injected intravenously only about 6 per cent of the administered dose was excreted unchanged in the urine. The remainder was rapidly broken down in the tissues and excreted as inorganic iodide. Hence, even if the thyroid hormone were quantitatively converted to diiodotyrosine in the tissues, no more than 6 per cent of the urinary iodide derived from hormone would be excreted as diiodotyrosine.

While the reviewer is prepared to grant the presence of minute amounts of thyroxine in urine, he is inclined to believe that the majority of the diiodotyrosine found by Rall represents some sort of an artifact. This supposition is strengthened by a study of the iodine compounds excreted after the intravenous administration of radioactive dl-thyroxine (2). Of the total radioactive iodine in the urine, about 85 per cent was inorganic iodide. Ten per cent behaved like thyroxine iodine and only 5 per cent or less appeared to behave like diiodotyrosine iodine. In a similar study, Myant and Pochin (122) found that after the intravenous administration of labelled thyroxine, 9 per cent of the radioactive iodine in the urine was thyroxine iodine as judged by butanol extraction. They did not attempt to identify diiodotyrosine but assumed that the remaining 91 per cent was inorganic iodide. In evaluating these reports it must be recalled that exogenous thyroxine may not be metabolized in quite the same manner as the normal thyroid hormone (see Section VI, B, 1).

Finally, the renal excretion of any considerable quantity of thyroxine may be doubted on theoretical grounds. In the blood stream thyroxine is so completely bound to plasma protein that it cannot be removed by ultrafiltration or prolonged dialysis (157, 174, 187). While this by no means precludes the existence of an exceedingly small concentration of unbound thyroxine (see Section XI, A), it probably prevents the kidney from removing more than traces of thyroxine from the blood stream. This argument, however, loses much of its force when it is realized that in a hyperthyroid patient with a renal plasma flow of 700 ml. per minute and a concentration of 14 microgm. of protein-bound iodine per 100 ml. of plasma, 141,000 microgm. of protein-bound iodine would flow through the renal blood vessels in 24 hours. It is obvious that removal of a very minute proportion of this could account for the quantities of thyroxine found by Rall in the urine of patients with hyperthyroidism.

In summary, the excretion of organic iodine in the urine can safely be disregarded in normal subjects. In hyperthyroidism the extent and nature of organic iodine in the urine require further investigation. Even in hyperthyroidism probably no great errors in calculation will occur if all of the urinary iodine is assumed to be inorganic iodide.

2. *Feces:* Early attempts to determine chemically the quantity of iodine normally excreted in the feces have been reviewed by Salter (162). It is doubtful that any of these analyses were sufficiently accurate to be trustworthy.

Since the original report by Krayer (86) many investigators have found that, after the parenteral administration of thyroxine, a very large proportion of the iodine may be excreted in the feces. Even with doses of radioactive l-thyroxine as small as 0.001 to 0.07 microgm., Gross and Leblond (63) found that rats excreted approximately 30 per cent of the administered radioactive iodine in the feces within 24 hours. Only a fraction of this was organic. The authors estimate that of the thyroxine present in the body of the rat at any one time less than $^1/_5$ is likely to be lost in the feces as thyroxine itself. With larger unphysiological doses (20–7,000 microgm.) a majority of the administered iodine appeared in the feces within 24 hours, and the proportion which behaved chemically like thyroxine was also much increased. In the rat, the appearance of thyroxine in the feces is due largely, if not entirely, to secretion of thyroxine by the liver into the bile (62, 86). Taurog and his collaborators (185) have recently demonstrated that in both dogs and rats the liver conjugates thyroxine, probably with glucuronic acid. Unless large doses are given, very little of the thyroxine in the bile is free. However, the conjugate is apparently hydrolyzed in the intestine, perhaps by the glucuronidase of the intestinal bacteria, so that only free thyroxine is found in the feces. The conjugate was also identified in the bile of rats given radioactive iodide, thus proving that endogenous thyroxine is also conjugated by the liver.

The possible importance of biliary excretion of thyroxine in man has also been suggested by two experiments in which the distribution of thyroxine after parenteral administration was studied. Albert and Keating (2) injected 1 mgm. of labelled thyroxine into a cretin who had been maintained in a euthyroid state with desiccated thyroid. During the following 8 days 41.3 per cent of the dose was excreted in the urine, the majority of it as iodide, while 11.5 per cent of the dose was excreted in the feces, most of it as precipitable iodine. Of the total excreted, therefore, approximately 22 per cent was fecal. In a similar study, Myant and Pochin (122) injected 80 microgm. of labelled thyroxine intravenously into 3 normal subjects, and recovered approximately 10 per cent of the radioactivity in the feces within a 3-day period. They did not attempt to determine the chemical nature of the fecal iodine. While these studies clearly demonstrate that in man, as in the rat, a very significant proportion of a dose of exogenous thyroxine may be lost from the body in the feces, they do not necessarily indicate that this is an important excretory route for endogenous thyroid hormone.

After the initial collection and excretion of a tracer dose of radioactive iodine has been completed, a comparison of radioactivity in urine and feces should permit calculation of the proportion of the endogenous hormone which is lost by fecal excretion. Unfortunately, the reviewer is not aware of any such studies with tracer amounts of radioactive iodine. However Dr. A. Stone Freedberg (47) has kindly supplied the following unpublished data from a patient with thyrotoxicosis who was given a therapeutic dose of radioactive iodine. Comparisons of urinary and fecal excretion were made on the 5th, 6th, 8th, 9th and 10th days following treatment. The mean urinary excretion was 4.66 per cent of the dose per day, while the mean fecal excretion was 0.67 per cent of the dose per day. Thus fecal loss constituted 12.5 per cent of the total excreted.

Because of the difficulty of measuring the loss of organic iodine in the feces directly, two indirect methods of calculation will now be presented. While the accuracy of these methods is probably not very great, they should at least indicate whether fecal loss of organic iodine is a *major* pathway of iodine metabolism in man.

If the intake of iodide is abruptly and completely stopped, the only remaining source of iodide in the urine will be breakdown of hormone in the tissues. The concentration of iodide in the blood will therefore decline towards an asymptotic value. When the asymptote has been reached, the quantity of iodide appearing in the urine should equal the quantity of iodide being returned to the iodide compartment from the tissues, multiplied by the proportion destined for renal excretion:

$$E_s = (1 - U)(H - F) = H(1 - U) - F(1 - U) \qquad 26$$

but from equation 15:

$$H(1 - U) = EU \qquad 15a$$

Hence:

$$E_s = UE - F(1 - U) \qquad 27$$

Solving for F:

$$F = \frac{UE - E_s}{1 - U} \qquad 28$$

It should therefore be possible to calculate the excretion of organic iodine in the feces from a comparison of the urinary excretion of iodide before and during a fast and from the uptake of radioactive iodine. Because of the rapidity with which the kidneys and thyroid clear iodide from the blood stream, the period during which the intake of iodine is eliminated need not be long. In normal subjects the asymptotic rate of excretion may be estimated from the iodine content of serial samples of urine collected every two or three hours during a 30-hour period of fasting (Figure 3). Much shorter periods should suffice for patients with hyperthyroidism. This is a great advantage since the validity of this method depends upon the assumption that starvation affects neither the uptake of iodide by the thyroid nor the rate of turnover of hormone in the tissues. It is quite unlikely that so brief a period of starvation would influence either of these processes.

If the excretion of organic iodine turns out to be negligible, equation 26 may also be used as a method for calculating the rate of secretion of the thyroid hormone: Solving equation 26 for H:

$$H = \frac{E_s}{(1 - U)} + F \qquad 29$$

and, if F is negligible:

$$H \cong \frac{E_s}{1 - U} \qquad 30$$

A second but much more cumbersome method for calculating F is based on the obvious fact that the rate of loss of labelled hormone from the thyroid gland ("biological decay") is dependent not only upon the excretion of iodide from hormone broken down in the tissues but also upon the loss of organic iodine from the body.

If sufficient time be allowed for equilibration after the administration of radioactive iodine, the specific activity of the hormone will be the same in the tissues as in the gland.[4] At that time:

$$24K_G = \frac{E^* + F^*}{Q_G^*} \qquad 31$$

Solving for F*:

$$F^* = 24K_G Q_G^* - E^* \qquad 32$$

Also, since F*, F, E* and (H − F) (1 − U) are all derived from the tissue hormone, F*/F and E*/(H − F) (1 − U) are both expressions for the specific activity and may be equated to each other:

$$\frac{F^*}{F} = \frac{E^*}{(H - F)(1 - U)}$$

or

$$\frac{F^*}{E^*} = \frac{F}{H(1 - U) - F(1 - U)} \qquad 33$$

Substituting in equation 33 the value for F* from equation 32:

$$\frac{24K_G Q_G^* - E^*}{E^*} = \frac{F}{H(1 - U) - F(1 - U)} \qquad 34$$

But from equation 15a,

$$H(1 - U) = UE \qquad 15a$$

Substituting this value in equation 34:

$$\frac{24K_G Q_G^* - E^*}{E^*} = \frac{F}{UE - F(1 - U)} \qquad 35$$

Solving for F:

$$F = \frac{UE(24K_G Q_G^* - E^*)}{24K_G Q_G^*(1 - U) + UE^*} \qquad 36$$

---

[4] This statement, which is repeated in Section VII-B, is sufficiently accurate for practical purposes, but it is not quite precise. The specific activity of hormone in the tissues will at first gradually increase as the specific activity in the gland steadily decreases (Figure 8). When the specific activity of hormone in the tissues attains its peak, for an instant it will exactly equal the specific activity of the hormone in the gland. Thereafter the specific activity in the tissues will begin to decline, but at first somewhat more slowly than does the specific activity in the gland. As equilibrium is approached, the ratio of specific activity in the tissues to specific activity in the gland will tend towards a constant value, but this asymptotic value will always be a little greater than unity. This is in accord with the general relationship described by Zilversmit *et al.* (210) for the specific activities of any radioactive compound and its immediate precursor. Equations based on equality of specific activity of hormone in thyroid and tissues are therefore *strictly* valid only when the specific activity of the hormone in the tissues is maximal.

This mode of calculation requires measurement of the uptake, the excretion of both stable and radioactive iodine, the biological decay and the quantity of radioactive iodine remaining in the thyroid gland. Were there no limit to the amount of radioactive iodine which can be given, all of these could be measured with considerable precision. However, in normal subjects the time which must elapse between the administration of a tracer dose and the attainment of equal specific activities in gland and tissues is probably too long to permit very accurate measurements of the daily excretion of radioactive iodine in the urine. However, the method might be useful in patients with hyperthyroidism in whom equal specific activities in tissues and gland should be attained sooner, and to whom it may be permissible to administer larger doses of radioactive iodine.

Unfortunately, the available data permit only a very crude estimate of the quantity of iodine excreted daily in organic form. The reviewer will hazard a guess that in the normal human it amounts to no more than 10 per cent of the iodine secreted as hormone. For purposes of illustration, 6 microgm. per day will be taken arbitrarily as the sum of urinary and fecal excretion of organic iodine in euthyroid subjects.

Comparatively little is known about pathological variations in the excretion of organic iodine. Kydd and Man (87) have found abnormally high concentrations of protein-bound iodine in the serum of patients with infectious hepatitis during the first four weeks of illness. They suggest that during the early phase of the disease, severe hepatic damage impairs the ability of the liver to destroy and excrete thyroxine which therefore accumulates in the plasma. This explanation seems improbable. Even if it were supposed that most of the thyroxine secreted is normally metabolized or excreted by the liver, the anterior pituitary, by decreasing its secretion of thyrotropic hormone, should easily be able to prevent any significant rise in the plasma concentration of thyroxine (59).

Peters and Man (131) found small quantities of protein-bound iodine in the urine of patients with nephrosis. They concluded that the loss of hormone was not sufficient to explain the low concentrations of protein-bound iodine in the serum of nephrotic subjects. This conclusion is also supported by Recant (153). However, to the extent that abnormal quantities of hormone are excreted in patients with proteinuria, there must be a compensatory increase in the activity of the thyroid gland for maintenance of a normal supply of hormone to the tissues. This may explain the unusually rapid and extensive uptake of radioactive iodine which was observed in some of the nephrotic subjects studied by Recant (see Figure 12).

E. *The Blood Flow through the Thyroid Gland:* In proportion to its weight, the normal thyroid gland is more richly supplied with blood than any other organ of the body except the lungs. In animals the flow has been estimated at 5.6 ml. per gram of tissue per minute (15), while in man, Means (107) estimates the flow at 4 ml. per gram of tissue per minute. Means also quotes a prescient remark by King (85) who, in 1836, said of the thyroid gland that, "Its nourishment does not seem to be the main intention of its vascular supply." It is now abundantly clear that "the main intention of its vascular supply" is to make up for the exceedingly minute *concentration* of inorganic iodide in the blood stream by presenting the gland with a *volume* of blood sufficiently large to provide the quantity of iodide needed daily for hormone manufacture.

The mean concentration of iodide ion in the plasma may be calculated by solving equation 4 for (I):

$$(\mathrm{I}) = \frac{\mathrm{E}}{1.44\mathrm{C_K}} \tag{4a}$$

This method of calculating the plasma concentration of the iodide ion is far more accurate than direct chemical estimation. The quantity of iodide excreted in the urine and the renal clearance of iodide may both be measured with considerable precision, while the actual concentration of iodide in plasma is too small to be measured accurately even with the highly sensitive analytical methods now in use. Since the normal kidneys clear iodide from the blood stream at the rate of approximately 33⅓ ml. per minute, and since the normal daily excretion of iodide is of the order of 144 microgm., the normal mean concentration of iodide ion in the plasma must be about 3 microgm. per liter. Hence, to obtain its daily requirement of 70 microgm., even the normal thyroid gland must extract the iodide from about 23 liters of plasma or 27 liters of whole blood each day.

Unfortunately, there seems to be a dearth of quantitative information on the maximum blood flow through the thyroid gland when it becomes hyperactive. It is common knowledge that the thyroid gland in Graves' disease and in hyperplastic goiter due to iodine deficiency is exceedingly vascular. How much of this increased vascularity is due to a simple increase in the size of the gland and how much to an increase in the blood flow per gram of tissue is not known. However, if the normal blood flow is approximately 5 ml. per gram of tissue per minute it seems unlikely that it could increase more than three- or four-fold and still leave room for active thyroid parenchyma.

A simple method for calculating the thyroid blood flow, which should at least be applicable to animals, is described in the following section.

F. *The Efficiency of Extraction of Iodide from the Blood Stream:* Like the daily secretion of hormone, the efficiency of extraction of iodide ion from the blood stream by the thyroid gland cannot be directly measured in the intact human being. However, it should be relatively simple to measure it in anesthetized animals or indeed in patients during operations on the thyroid gland. Samples of arterial blood flowing to, and venous blood flowing from, the gland could be collected after the administration of a tracer dose of radioactive iodine. The arteriovenous difference in the concentration of radioactive iodine would provide a direct measure of the efficiency of extraction:

$$\mathrm{P} = \frac{(\mathrm{I})^*_{\mathrm{A}} - (\mathrm{I})^*_{\mathrm{TV}}}{(\mathrm{I})^*_{\mathrm{A}}} \tag{37}$$

If time enough were allowed for the radioactive iodide to approach distribution equilibrium, venous blood from another region could be substituted for arterial blood.

Apparently Pochin (137) is the only investigator who has used this method. In six patients undergoing subtotal thyroidectomy for non-toxic adenoma the concentration of radioactive iodide in blood from a vein draining the more normal thyroid lobe was compared with the concentration in blood from an arm vein. The mean extraction efficiency was 0.2.

This technique could also be employed for the calculation of the thyroid blood flow. Solving equation 1 for ϕ:

$$\phi = \frac{1.19C_G}{M_G P} \tag{1a}$$

It is obvious that:

$$\frac{C_G}{C_K} = \frac{U}{1 - U} \tag{38}$$

Hence:

$$C_G = \frac{UC_K}{1 - U} \tag{39}$$

Substituting this value for C_G in equation 1a:

$$\phi = \frac{1.19UC_K}{(1 - U)PM_G} \tag{40}$$

Since U and C_K are easily measured, and since P can be calculated by equation 37, equation 40 provides a means of studying the total thyroid blood flow, ϕM_G, under various conditions. If the thyroid gland were then removed and weighed, the blood flow per gram of gland per minute, ϕ, could also be calculated. Assuming normal values for the thyroid clearance and the weight of the thyroid gland, Pochin estimated that the mean blood flow in the six patients whom he studied was 6 ml. per gram of thyroid per minute.

An estimate of the efficiency of extraction may be derived from equation 8. Solving this equation for P,

$$P = \frac{1.19C_K H}{\phi M_G(\text{In} - F)} \tag{8a}$$

Normal values for all of the factors in this equation have already been estimated with the exception of M_G, the mass of the normal thyroid gland. For this we may accept the value of 20 gm. given by Means (107). Substituting these values in equation 8a:

$$P = \frac{(1.19)(33\ 1/3)(70)}{(5)(20)(150-6)} = 0.19$$

This is almost identical with the mean value given by Pochin. It is evident, therefore, that even in regions of relative iodine abundance the normal thyroid gland must remove almost 20 per cent of the iodine from the blood which flows through it. Even if the efficiency could increase to 100 per cent in response to a decreased iodine supply or an increased secretion of hormone, the clearance of iodide from the blood stream by the thyroid gland would increase only about five-fold. The ability of the extraction efficiency to adapt itself to demands for *increased* thyroid function is therefore quite limited. On the other hand, the extraction efficiency can, and undoubtedly does *decrease* almost without limit in response to an excessive supply of iodide (see Section X, B).

The efficiency of the thyroid gland in extracting iodide from the blood stream is dependent in part upon its ability to maintain a concentration gradient of the iodide ion between gland and blood stream, and in part upon its ability to oxidize inorganic iodide and incorporate it rapidly into tyrosine. Since both of these mechanisms may be specifically impaired by certain drugs, further discussion of them will be deferred to the section on the influence of pharmacological agents on iodine metabolism and thyroid function.

VII. FACTORS WHICH INFLUENCE THE DISTRIBUTION OF RADIOACTIVE IODINE

Thus far the discussion has centered about iodine metabolism and thyroid function at equilibrium, and simple algebraic equations have sufficed. But the equilibrium is a *dynamic* equilibrium and for the study of its dynamics it is necessary to consider the distribution of a tracer dose of radioactive iodine as it traverses the various metabolic pathways leading to and from the several compartments. Mathematical treatment of the behavior of radioactive iodine within the body therefore requires the use of differential equations to describe the rate of change of the quantity of radioactive iodine in each compartment with the passage of time after the administration of a tracer dose. This rate of change will depend upon the difference between the rate at which radioactive iodine is entering the compartment and the rate at which it is leaving the compartment. Hence at any time after a tracer dose:

$$\frac{dQ_I^*}{dt} = K_{BI}Q_B^* - (K_{IE} + K_{IG})Q_I^* \qquad 41$$

$$\frac{dQ_G^*}{dt} = K_{IG}Q_I^* - K_{GB}Q_G^* \qquad 42$$

$$\frac{dQ_B^*}{dt} = K_{GB}Q_G^* - (K_{BI} + K_{BF})Q_B^* \qquad 43$$

The initial conditions assumed (Section III, B, 3) being:

$$\text{When } t = 0, Q_I^* = 1, Q_G^* = 0, Q_B^* = 0$$

Since equations 41, 42 and 43 are interdependent, a rigorous mathematical description of the distribution of radioactive iodine would require the simultaneous solution of all three equations which is by no means easy. The reader is referred to the paper by Oddie (126) for a discussion of some of the complexities involved. Of the three equations, only the first two are easily applied. During the first few hours after the administration of a tracer dose, the distribution will depend chiefly on K_{IE} and K_{IG} since, in proportion to these, all of the other rate constants are usually small enough to be neglected. Hence:

$$\frac{dQ_I^*}{dt} \cong -(K_{IE} + K_{IG})Q_I^* \qquad 41a$$

and

$$\frac{dQ_G^*}{dt} \cong K_{IG}\,Q_I^* \qquad 42a$$

Although Brownell (18) has suggested an approximate method for the calculation of Q_B^* from the various rate constants, it involves the assumption that the loss of labelled hormone from the gland is governed throughout by K_G. This assumption is not supported by the observed behavior of radioactivity in the gland (see Section IX, B).

Thus far, the problem of the distribution of radioactive iodine has been discussed as though the rate constants were known, and the quantities of radioactive iodine in each compartment were to be calculated from them. In practice, a mathematical analysis would be most useful for calculating the rate constants from serial observations of the quantities. In view of the difficulty of rigorous mathematical treatment, Brownell's proposal that an analogue computer be used in the analysis of data from tracer experiments is attractive (18).

The dynamic behavior of radioactive iodine will obviously be affected by all of the factors discussed in previous sections. In addition, however, it will be affected by a group of factors which have no direct influence on thyroid activity. The factors hitherto discussed are for the most part represented by the arrows in Figure 2. However, rates of transfer of radioactive iodine are equally dependent upon the sizes of the cubes in Figure 2. At equilibrium, the rate constant of transfer from a compartment along a given pathway is equal to the quantity of iodine traversing that pathway per hour divided by the quantity of iodine present in the compartment. The quantities of iodine contained by each compartment must now be discussed.

A. *The Iodide Compartment:* The total quantity of iodide ion in the body is the product of the iodide concentration in the plasma and the volume of distribution of the iodide ion.

$$Q_I = (I)V_I \qquad 44$$

It has already been pointed out that the most satisfactory method for calculating the iodide concentration is by the use of equation 4a:

$$(I) = \frac{E}{1.44C_K} \qquad 4a$$

Although it is possible to calculate the volume of distribution from equation 11, this is likely to be untrustworthy, since the theoretical value for the initial concentration of radioactive iodine in the plasma must be obtained by extrapolation of a logarithmic plot of observed plasma concentrations to zero time. Because of the time needed for absorption and distribution of the radioactive iodine, the extrapolated value is likely to be in error, particularly when the radioactive iodine is administered by mouth. It is therefore preferable to solve equation 9 for V_I and calculate the volume of distribution from the rate constant of excretion and from the renal clearance:

$$V_I = \frac{6C_K}{100K_{IE}} \qquad 45$$

The calculation of C_K has already been discussed. K_{IE} may be calculated from equation 10, provided that $K_{IE} + K_{IG}$ can be measured.

The integral of equation 41a is:

$$Q^*_{I_t} = Q^*_{I_0}\, e^{-(K_{IE} + K_{IG})t} \qquad 46$$

Hence

$$\ln Q^*_{I_t} = \ln Q^*_{I_0} - (K_{IE} + K_{IG})t \qquad 47$$

Solving for $K_{IE} + K_{IG}$:

$$K_{IE} + K_{IG} = \frac{\ln Q^*_{I_0} - \ln Q^*_{I_t}}{t} \qquad 48$$

It is evident, therefore, that $K_{IE} + K_{IG}$ is simply the slope of the straight line obtained when the natural logarithm of the quantity of radioactivity remaining in the iodide compartment is plotted against time. If the volume of distribution remains constant, the concentrations of radioactive iodide may be used in equation 48 instead of the total quantities.

Keating and his collaborators have pointed out that the rate of disappearance of radioactive iodine from the blood stream ($K_{IE} + K_{IG}$) may be estimated from its rate of appearance in the urine, its rate of collection by the thyroid gland, or its rate of disappearance from any indifferent region of the body such as the thigh, as well as from the rate of decrease of its concentration in the plasma (95).

Keating and Albert (80) have calculated that the volume of distribution of inorganic iodide in six euthyroid subjects was 26.0 ± 3.5 liters or 35 per cent of the body weight. Similar values were found in six patients with myxedema and three with adenomatous goiter without hyperthyroidism, the weighted mean for the whole group of 15 patients without hyperthyroidism being 24 liters or 34 per cent of the body weight. In 16 patients with exophthalmic goiter the apparent volume of distribution was somewhat larger, averaging 31.2 liters or 53 per cent of the body weight.

Myant and his collaborators (119) proposed a somewhat different method for the calculation of the volume of distribution. By subtracting from the administered dose the quantities of radioactive iodine which had already been accumulated by the thyroid gland and excreted in the urine, they obtained the quantity remaining in the body and presumably distributed throughout the iodide compartment. The amount remaining, divided by the concentration of radioactive iodide in the plasma gave the volume of distribution. At any given moment (prior to the appearance of labelled hormone in the blood stream):

$$V_I = \frac{1 - (Q^*_{G_t} + Q^*_{E_t})}{(I)^*_t} \qquad 49$$

The mean volume of distribution of iodide calculated in this manner in 14 healthy normal subjects was 18 liters, or 28 per cent of the body weight, at 1 hour after the administration of radioactive iodine intravenously. However, the iodide space appeared to increase further with the passage of time so that at 6 hours it was approximately 25 liters or 39 per cent of the body weight. The latter estimates agree well with those of Keating. The authors discuss a number of possible explanations for the apparent slow increase in the volume of distribution of iodide after the first two hours. In another paper Myant and his collaborators (123) comment as follows, "It seems reasonable to assume, therefore, that the radioiodide diffuses through a volume comparable with the body water after some hours." The reviewer does not feel that this conclusion is substantiated either by the data presented by Myant or by the more extensive studies of Keating's group.

If the expression for (I) of equation 4a and the expression for V_I of equation 45 be substituted in equation 44, the following very simple equation for the mean quantity of iodide in the iodide compartment is obtained.

$$Q_I = \frac{E}{24K_{IE}} \qquad 50$$

As a matter of fact, equation 50, and the analogous equations to be introduced later, need not be derived in this manner, since they are simply algebraic expressions of the definition of a rate constant at equilibrium. A mean normal value for K_{IE} may be obtained from the exceedingly extensive data of Keating and his collaborators (81). Excluding patients with renal, cardiac or thyroid disorders, or with debilitating diseases, such as carcinoma or Addison's disease, there remains a group of 202 euthyroid subjects in which the mean renal excretion rate, K_{IE}, was 0.072 per hour. Substituting this value, and the estimate of 144 microgm. per day for the normal excretion of iodide, in equation 50, one obtains an estimate of 84 microgm. of iodide in the entire iodide compartment. If the mean iodide concentration is taken as 3 microgm. per liter, the volume of distribution by equation 44 would be approximately 28 liters, which is in good agreement with the estimates quoted above. For illustrative purposes, a value of 25 liters will be taken as the mean volume of distribution of the iodide ion and a value of 75 microgm. as the mean quantity of iodide ion within the body in normal man.

The volume of distribution of the iodide ion is considerably larger than the volume of distribution of chloride, bromide or thiocyanate. This does not necessarily indicate that the iodide ion can penetrate the cells of most tissues more readily than the other ions. Iodide concentrated in the thyroid gland and in the secretions of the salivary and gastric glands may well account for the larger volume of distribution. For example, if the concentration of iodide in gastric juice is 30 times the concentration in plasma, 100 ml. of gastric juice will contribute 3 liters to the volume of distribution of iodide. Furthermore, the thyroid gland may, under certain circumstances, contain more iodide ion than all of the rest of the body together (see Section IX, C).

Pathological expansion of the iodide compartment occurs in patients with edema. Not only is the volume of distribution of the iodide ion increased, but there may also be an unusual delay in the attainment of distribution equilibrium when a tracer dose of radioactive iodine is given. Myant and his coworkers (119) have found that the concentration of radioactive iodide in ascitic fluid, or in edema fluid draining from Southey's tubes, increases rather slowly and does not become equal to the plasma concentration until five or six hours after the intravenous administration of a tracer dose of radioactive iodine. The assumption of instantaneous and even distribution of radioactive iodine throughout the iodide compartment is patently invalid in patients with large reservoirs of edema fluid.

B. *Organic Iodine in the Thyroid Gland:* After the administration of a tracer dose of radioactive iodine, the specific activity of hormone in the tissues will gradually approach the specific activity of hormone in the thyroid gland. When the specific activities have become equal:

$$\frac{Q_G^*}{Q_G} = \frac{(B)^*}{(B)} = \frac{E^*}{(1 - U)(H - F)} \qquad 51$$

Hence:

$$Q_G = \frac{Q_G^*(B)}{(B)^*} \qquad 52$$

and:

$$Q_G = \frac{Q_G^*(1 - U)(H - F)}{E^*} = \frac{Q_G^*[H(1 - U) - F(1 - U)]}{E^*} \qquad 53$$

but:

$$H(1 - U) = UE \qquad 15a$$

Therefore:

$$Q_G = \frac{Q_G^*[UE - F(1 - U)]}{E^*} \qquad 54$$

Or, if F is negligible:

$$Q_G = \frac{Q_G^* UE}{E^*} \qquad 55$$

According to equation 52 it should be possible to calculate the quantity of organic iodine in the thyroid gland from the specific activity of protein-bound iodine in the blood stream. However, with tracer doses of reasonable size, the specific activity is likely to be too low for equation 52 to be of any practical value. Equation 55 should be more useful since it may be possible to concentrate the radioactive iodide from an entire 24-hour sample of urine and measure it with some precision.

Another method based upon specific activity is advantageous since it provides larger quantities of radioactive iodine in the urine. From equation 23 it is evident that when equal specific activities of the hormone in thyroid and tissues have been attained and synthesis of hormone is completely blocked:

$$\frac{Q_G^*}{Q_G} = \frac{H^*}{H} = \frac{E_{b1}^* - E^*}{E_{b1} - E} \qquad 56$$

Solving for Q_G:

$$Q_G = \frac{(E_{b1} - E)Q_G^*}{E_{b1}^* - E^*} \qquad 57$$

This method has actually been used to calculate the quantity of iodine in the thyroid glands of patients with goiter due to iodine deficiency (179). Equation 57 is valid even if the blocking agent accelerates the rate of loss of radioactive iodine from the thyroid gland (see Section VI, B, 3) since the specific activity should not be altered thereby.

A second mode of calculation is based not upon specific activity, but upon the rate at which labelled hormone is lost from the thyroid gland. By definition:

$$Q_G = \frac{H}{24K_{GB}} \qquad 58$$

If the thyroid gland could be instantaneously and uniformly labelled with radioactive iodine, K_{GB}, the rate constant of transfer of hormone from the gland to the tissues, could theoretically be obtained from the *initial* rate of loss of radioactivity from the gland, *i.e.*, the rate of loss before appreciable quantities of radioactive iodine begin to return to the gland from breakdown of labelled hormone in the tissues. In fact, however, the initial accumulation of radioactive iodide is not instantaneous, and is likely to overlap somewhat with the return of iodide from hormone synthesized and secreted during the early stages of uptake. This mode of calculation will tend to underestimate the true value for K_{GB} and will therefore lead to an overestimate of the quantity of organic iodine in the thyroid.

An alternative method depends on measurement of the rate of loss of radioactivity from the thyroid when equality of specific activities has been attained in gland and tissues. The rate of loss will then no longer be proportional to H but rather to:

$$(1 - U)(H - F) + F = H(1 - U) + UF \qquad 59$$

and therefore:

$$Q_G = \frac{H(1 - U) + UF}{24K_G} \qquad 60$$

Where K_G is the rate constant for the *net* loss of radioactivity from the gland. But from equation 15a:

$$H(1 - U) = UE \qquad 15a$$

Hence:

$$Q_G = \frac{U(E + F)}{24K_G} \qquad 61$$

and if F is negligible:

$$Q_G = \frac{UE}{24K_G} \tag{62}$$

As far as the reviewer is aware, no one has attempted to apply this method for the calculation of the quantity of organic iodine in the thyroid gland. However, the method may be illustrated by using the data of Burns and his coworkers (21) who measured the loss of radioactivity from the thyroid glands of two normal subjects during a period of 50 days. Through the kindness of Professor W. A. Fish, the data from one of these subjects are reproduced in Figure 4. In this subject the biological half-life was calculated to be 94 days (21). The value of K_G calculated from this figure is 0.000306. The uptake was 0.417. Using these figures, and the assumed values of 144 microgm. per day for E and 6 microgm. per day for F, the quantity of organic iodine in the thyroid gland calculated by equation 61 is approximately 8,500 microgm., a value very close to the normal mean.

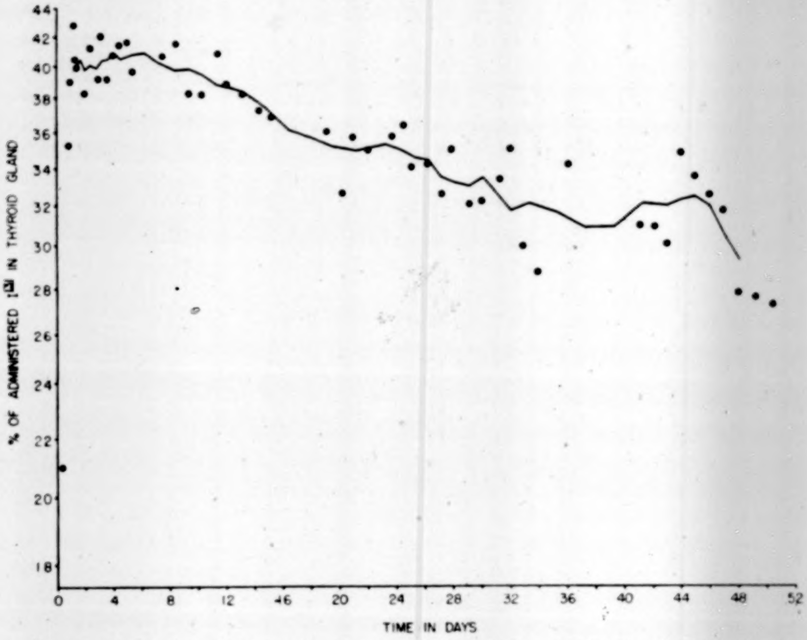

FIG. 4. The biological decay of radioactive iodine in the thyroid gland of a euthyroid subject studied by Burns *et al.* (21 (Subject No. 1)). The dose of radioactive iodide given at time zero was 100 microcuries. The per cent of the dose remaining in the thyroid has been corrected for physical decay and is plotted on a logarithmic scale against time. The solid line represents a five-point moving average. Note the very slow rate at which radioactive iodine is lost from the gland.

In his monograph, McClendon (101) has tabulated the results of two extensive series of analyses in which the iodine content of the normal human thyroid was determined. In 30 glands analyzed by Remington, McClendon and their co-workers in Charleston, South Carolina, the average quantity of iodine per gland was 8,530 microgm. In 52 glands analyzed by Leland and Foster in New York, the average iodine content per gland was 7,444 microgm. The weighted mean of these two series is 7,840 microgm. We may conveniently take 8,000 microgm. as the mean quantity of organic iodine in the normal human thyroid.

McClendon's tabulation indicates that even in normal individuals considerable variation in the iodine content of the thyroid may be expected. Moreover, the administration of iodide will tend to increase the quantity of stored hormone. In Graves' disease and in iodine deficiency the concentration of hormone in the gland may be very greatly reduced although the total quantity may be decreased only moderately because of the increase in the size of the gland. These variations in iodine content will profoundly influence the rate at which radioactive iodine is turned over in the thyroid.

C. *Organic Iodine in Extrathyroidal Tissues:* Surprisingly little attention has been given to the influence of organic iodine in the peripheral tissues on the behavior of radioactive iodine. The methods which will now be described for the calculation of the quantity of extrathyroidal hormone are entirely analogous to those already used for calculation of the quantities of iodine in the iodide compartment and in the thyroid gland.

It is obvious that:

$$Q_B = (B)V_B \qquad 63$$

The concentration of organic iodine in the plasma, (B), is easily measured by determining the concentration of protein-bound iodine. The mean normal value may be taken as 50 microgm. per liter. Unfortunately, however, there are very few estimates available of the volume of distribution of the thyroid hormone, and even these are derived from studies of the distribution of exogenous thyroxine, rather than endogenous hormone. It is generally agreed (2, 122, 153) that after intravenous injection the concentration of thyroxine in the plasma decreases at a moderately slow rate for the first six to twelve hours, and thereafter at a very slow rate. The initial decrease is presumably due to the diffusion of thyroxine from the blood stream to the tissues and the subsequent very gradual decrease is presumably due to the gradual breakdown and excretion of thyroxine. From the second portion of the curve for thyroxine in the plasma Albert and Keating (2) calculated that the volume of distribution of thyroxine was 11.1 liters, or 19.3 per cent of the body weight, in a cretin maintained in a euthyroid state with desiccated thyroid. This volume of distribution seems surprisingly small. In a similar study Myant and Pochin (122) investigated the distribution of racemic or levo-thyroxine labelled with radioactive iodine in six normal subjects. The mean volume of distribution was 13 per cent of the body weight at one hour, 22.8 per cent of the body weight at 6 hours, and 32.6 per cent of the body weight at 24 hours after intravenous administration. As nearly as may be judged from these figures, distribution was practically complete at 24 hours.

For an individual weighing 70 kgm. the volume of distribution would therefore be approximately 23 liters. Taking the concentration of organic iodine as 50 microgm. per liter, this would give a total of 1,150 microgm. of organic iodine in the extrathyroidal tissues.

A second mode of calculation makes use of an equation entirely analogous to equations 50 and 58. By definition:

$$Q_B = \frac{H}{24(K_{BI} + K_{BF})} \qquad 64$$

Theoretically, if the secretion of hormone from the thyroid gland could be abruptly eliminated, the rate constant of disappearance of hormone from the tissues, $K_{BI} + K_{BF}$, could be calculated from the rate at which the protein-bound iodine of the serum decreased. This cannot be done in the intact human being. Alternatively, the rate of decrease of protein-bound iodine could be measured after abrupt withdrawal of thyroid from patients with myxedema, but such studies have not been systematically performed. The reviewer is forced to base his estimate for the peripheral rate of turnover of thyroid hormone on fragmentary evidence which he has culled from his own records and those of others. It should be emphasized that not one of these bits of evidence could survive careful scrutiny. Taken together, they suggest that the normal biological half-life of hormone in the tissues is very roughly ten days, and that $K_{BI} + K_{BF}$ is about 0.0029. If the normal rate of hormone secretion be taken as 70 microgm. of thyroxine iodine per day, substitution of this value in equation 64 yields an estimate of approximately 1,000 microgm. of organic iodine in extrathyroidal tissues.

Trunnell and his collaborators (194) estimate that the biological[5] half-life of radioactive iodine (presumably organic) in the tissues is 13.3 days. This estimate was obtained from studies in which doses of radioactive iodine large enough to destroy thyroid function were administered, but the secretion of hormone was probably not eliminated abruptly. Indeed, for several days such large doses may accelerate the loss of organic iodine from the gland and much of this organic iodine may be thyroglobulin rather than thyroxine. Under these circumstances it is difficult to evaluate the significance of the half-life.

The "balance-sheet" method used by Myant (see Section VII, A) for calculating the volume of distribution of inorganic iodide could also be applied to the volume of distribution of organic iodine. At any time after completion of the initial collection and excretion of radioactive iodide:

$$V_B = \frac{1 - (Q^*_{G_t} + Q^*_{E_t} + Q^*_{F_t})}{(B)^*_t} \qquad 65$$

The accuracy of this method is limited by the precision with which $Q^*_{E_t}$, $Q^*_{F_t}$ and particularly $(B)^*_t$ can be measured after a tracer dose of radioactive iodine.

---

[5] Actually Trunnell *et al.* report that the "biologic half-life" is 5 days. By this they mean the total loss due to both radioactive decay (half-life 8 days) and loss from tissues (half-life 13.3 days). The reviewer feels that it is less confusing to reserve the term "biological half-life" for the purely biological process of turnover in the tissues.

However, the data of Goldsmith and his coworkers (52) suggest that the method might easily be used in patients with hyperthyroidism.

A direct estimate of the volume of distribution of organic iodine could be obtained from chemical analysis of serum and tissues removed from euthyroid humans at autopsy. Such studies do not appear to have been made. Carr (23) has recently completed a careful analysis of the distribution of protein-bound iodine in the tissues of normal dogs. The results indicate that the volume of distribution of the thyroid hormone is considerably larger than the volume of extracellular fluid. Rall and his coworkers (143) studied the distribution of radioactive iodine in the blood and tissues 56 hours after the oral administration of 63 millicuries of radioactive iodine to a patient with metastatic carcinoma of the thyroid. In most tissues such as bone marrow, brain, liver, kidneys, muscle, spleen and gastrointestinal tract the concentration of radioactive iodine which was precipitable with zinc hydroxide and therefore presumably organic, was equal to or greater than the concentration of precipitable iodine in the blood. This would imply a volume of distribution considerably higher than the estimates derived above. Again, these data must be interpreted cautiously because of the destructive action of a large dose of radioactive iodine on the thyroid gland.

In the absence of more exact information the reviewer estimates that the normal quantity of organic iodine in the extrathyroidal tissues is 1200 microgm., and that the volume of distribution of organic iodine in extrathyroidal tissues is 24 liters. These estimates are based largely upon the studies of Myant and Pochin. The normal rate of turnover of thyroid hormone in the tissues may now be calculated from equation 64:

$$Q_B = \frac{H}{24(K_{BI} + K_{BF})} \qquad 64$$

$$1200 = \frac{70}{24(K_{BI} + K_{BF})}$$

$$K_{BI} + K_{BF} = 0.00243$$

which corresponds to a half-life of 11.9 days. The probability that $K_{BI} + K_{BF}$ varies with the concentration of thyroid hormone will be discussed in Section XI, A.

In Table 3 are summarized all of the normal values which have been discussed in the preceding sections, together with other quantities which have been calculated from them. While most of the values are certainly of the correct order of magnitude, the values for V_B and F are highly tentative and may require drastic revision as new evidence accumulates. Perhaps the best that can be said of the tabulated figures is that they are internally consistent. For comparison the table also contains values which are probably typical of certain common alterations in thyroid function. These and certain other changes in thyroid activity will now be considered.

VIII. PHYSIOLOGICAL VARIATIONS IN IODINE METABOLISM AND THYROID FUNCTION

In comparison with most of its endocrine partners, the thyroid gland is remarkably phlegmatic. Its sole duty seems to be the maintenance of an optimal

TABLE 3

Illustrative values of the quantities discussed in the text

QUANTITY*	UNITS	CONDITION				
		Normal (Fig. 2)	Acute iodine deficiency (Fig. 5)	Chronic iodine deficiency (Fig. 6)	Thyrotoxicosis (Fig. 7)	Thyrotoxicosis. Acute block of hormone synthesis (Fig. 9)
H	microgm./day	70	70	70	597	597
In	microgm./day	150	15	15	250	250
E	microgm./day	144	53.1	9	199	796
F	microgm./day	6	6	6	51.1	51.1
Q_I	microgm.	75	27.8	4.69	103	664
Q_G	microgm.	8,000	8,000	3,000	5,500	4,310
Q_B	microgm.	1,200	1,200	1,200	3,360	3,360
V_I	liters	25	25	25	25	40
M_G	gm.	20	20	120	60	60
V_B	liters	24	24	24	24	24
(I)	microgm./l.	3.0	1.11	0.19	4.14	16.6
(G)†	microgm./kgm.	400,000	400,000	15,000	91,700	71,800
(B)	microgm./l.	50	50	50	140	140
C_G	ml./min.	16.2	16.2	259	100	0
C_K	ml./min.	33.3	33.3	33.3	33.3	33.3
U	proportion	0.327	0.327	0.886	0.750	0.000
1-U	proportion	0.673	0.673	0.114	0.250	1.000
K_{IG}	proportion/hr.	0.0389	0.0389	0.622	0.240	0.000
K_{IK}	proportion/hr.	0.0800	0.0800	0.0800	0.0800	0.0500
K_{GB}	proportion/hr.	0.000364	0.000364	0.000972	0.00452	0.00577
K_G	proportion/hr.	0.000255	‡	0.000185	0.00142	‡
K_{BI}	proportion/hr.	0.00222	0.00222	0.00222	0.00676	0.00676
K_{BF}	proportion/hr.	0.000208	0.000208	0.000208	0.000635	0.000635
Biol. ½ life of						
I^{131} in I	hrs.	5.8	5.8	1.0	2.2	13.9
I^{131} in G (initial)	days	79	79	30	6.4	5.0
I^{131} in G (final)	days	113	‡	156	20.3	‡
I^{131} in B	days	11.9	11.9	11.9	3.9	3.9

* For the definition of the symbols used in this table, see Section V.

† The concentration of organic iodine in the thyroid gland expressed as microgm. per kgm.

‡ These values cannot be calculated since the conditions assumed are not equilibrium conditions.

concentration of thyroid hormone in the tissues (145). Physiological variations in thyroid function occur for the most part only when an increased rate of utilization of hormone in the tissues demands a compensatory increase in the rate of secretion or when the manufacture of a normal daily quantity of hormone is impeded.

A. *Sex:* There seems to be little difference in thyroid activity between the sexes as indicated by serum protein-bound iodine (148) or uptake of radioactive iodine (139).

B. *Age:* Except during pregnancy the concentration of hormonal iodine in the blood stream remains remarkably constant from birth to death. While there may be a downward trend with advancing age, the decrease is small (195). However, there is at least circumstantial evidence that the rate at which the tissues utilize thyroxine does not remain constant throughout the life cycle. In regions of iodine deficiency the increased incidence of goiter in children at puberty and particularly in adolescent girls has been abundantly documented (98, 101, 107). Although this strongly suggests an increase in the requirement for thyroid hormone during sexual maturation, objective proof is lacking. In old age, Perlmutter and Riggs (128) found a significant decrease in the rate at which the thyroid gland accumulates radioactive iodine. The decrease was most striking in females beyond the menopause and, judging from a small number of determinations, was not associated with any significant decline in the concentration of protein-bound iodine in the serum. The authors suggested that in senescence the activity of the thyroid gland decreases because of decreased peripheral utilization of the thyroid hormone. Unfortunately, these investigators depended solely upon the the *rate* of accumulation as measured by the "accumulation gradient". Even if there were no change in thyroid function, some decrease in the accumulation rate in elderly individuals might be expected because of the tendency for renal function to decline with advancing years (176). More significant is the report of Quimby and her collaborators (139) that the uptake of radioactive iodine decreases slightly in successive decades. Thyroid function in adolescence and senility should be reinvestigated using the methods for the calculation of the daily secretion of thyroid hormone which have been described.

C. *Pregnancy:* So far as is known, pregnancy is the only physiological state regularly accompanied by an increase in the concentration of thyroid hormone in the blood stream. The protein-bound iodine of the serum commonly falls in the range of 7 to 10 microgm. per cent, values which would be characteristic of mild hyperthyroidism in non-pregnant individuals (74, 96, 132). But in pregnancy the increased concentration of hormone in the plasma seems to be necessary for the maintenance of a *normal* metabolic state since it is fully established by the sixteenth week, long before the occurrence of the hypermetabolism which is characteristic of the third trimester. Danowski has mentioned the possibility that the increased concentration of protein-bound hormone might be explained by an increase in the protein groups responsible for binding thyroxine (36). If this factor alone were responsible, thyroid function itself should be normal. Yet there is good evidence that in pregnancy as in adolescence, hyperplasia of the thyroid gland is prone to occur, especially in regions of iodine deficiency. Furthermore, an increase in the avidity of the thyroid gland for radioactive iodine has been noted in pregnant rabbits (75). The reaction of the thyroid gland to pregnancy awaits clarification by further studies.

D. *Exposure to Cold:* In small laboratory animals such as the rat exposure to cold produces thyroid enlargement and increases the quantity of exogenous thyroid hormone required for the prevention of goiter due to the administration of thiouracil derivatives. Uotila (196) found that section of the pituitary stalk would prevent the hypertrophy of the thyroid due to cold exposure but not the compensatory hypertrophy due to subtotal thyroidectomy. This would suggest that the thyroid enlargement is due to primary stimulation of the anterior pituitary gland. However, the recent demonstration that the protein-bound iodine in the serum not only fails to rise but may actually decrease slightly when rats are exposed to cold strongly suggests that the anterior pituitary is stimulated by an increased rate of withdrawal of hormone from the blood stream, and that the thyroid hyperplasia is no more than a compensatory response to an increase in the rate of turnover of the thyroid hormone in the tissues (44, 145). In man, chronic exposure to cold in the Arctic also caused no increase, and possibly a slight decrease, in protein-bound iodine, without any significant change in the basal rate of metabolism (54). While these results provide no evidence for an effect of a cold environment on thyroid function in man, they by no means exclude the possibility that, during exposure to cold, the tissues may require an increase in the supply of thyroid hormone for maintenance of a *normal* metabolic rate.

E. *Other Stresses:* Violent exercise (148), inanition due to anorexia nervosa (131), a low protein intake (Kempner rice diet) (34), and a prolonged oversupply of adrenomedullary hormone in a patient with pheochromocytoma (154) are all apparently without effect on thyroid function as measured by the concentration of protein-bound iodine in the serum. In addition the accumulation of radioactive iodine is normal in anorexia nervosa (81, 200). However, the profound effect which adrenocortical hormones may have upon thyroid function (1, 14, 46, 76, 113, 130) suggests that the possible influence of stresses strong enough to stimulate the adrenal cortex (17) should be carefully investigated in man.

F. *Iodine Deficiency:* The effect of a decreased intake of iodine upon iodine metabolism and thyroid function illuminates many of the concepts developed in the preceding sections. The following brief description is illustrated by Figures 5 and 6, which should be compared with Figure 2.

Consider a euthyroid individual living in a region of iodine abundance, and in equilibrium when his mean intake of iodide is 150 microgm. per day (Figure 2). Immediately upon taking up residence in a region of severe iodine deficiency he will be subjected to the state of negative iodine balance illustrated by Figure 5. If the daily intake of iodine is reduced from 150 to 15 microgm. the concentration of iodide in the iodide compartment will rapidly decrease to a level which, as in starvation, will be determined largely by the quantity of iodide returned from breakdown of hormone in the tissues (see Section VI, D, 2). Since the renal clearance of iodide does not change (179), iodide excretion will exceed intake and the thyroid gland will enter a long period of attrition during which its stores of hor-

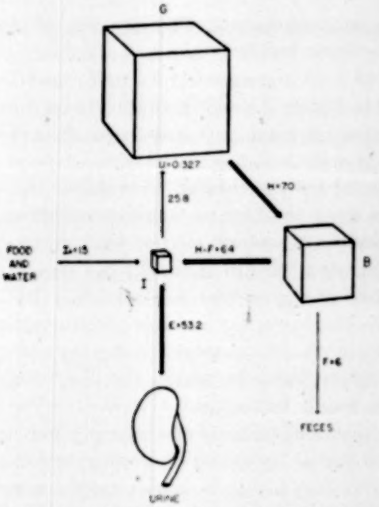
G
U=0.327
H=70
25.8
FOOD AND WATER
$I_E=15$
H-F=64
B
I
E=53.2
F=6
FECES
URINE

FIG. 5. A diagram of iodine metabolism in the euthyroid subject of Figure 2 soon after he has moved to a region of iodine deficiency (Table 3, column 4). The construction of the diagram has been explained in the legend of Figure 2. Note the decrease in size of compartment I.

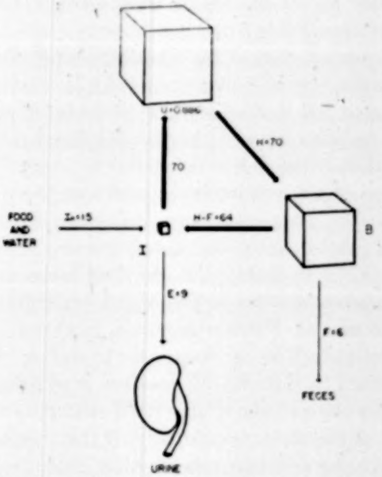
U=0.086
H=70
70
FOOD AND WATER
$I_E=15$
H-F=64
B
I
E=9
F=6
FECES
URINE

FIG. 6. A diagram of iodine metabolism in the euthyroid subject of Figure 5 after iodine balance has been attained in the face of a reduced intake of iodide (Table 3, column 5). The construction of the diagram has been explained in the legend of Figure 2.

Note that compartment G has become smaller, and that compartment I has shrunk to minute size. The importance of reutilization of iodide released from the breakdown of hormone in compartment B is evident from the figure.

mone gradually approach exhaustion. The duration of this period will depend upon the difference between iodide intake and excretion, the rate at which hormone is being secreted, and the quantity of preformed hormone. The state of imbalance illustrated in Figure 5 could theoretically continue for 181 days before the last reserves of stored hormone are exhausted. However, it is probable that as the concentration of organic iodine in the thyroid gland decreases, it becomes more and more difficult[6] for the thyroid to mobilize the quantity of hormone which it must secrete daily in order to maintain a normal concentration in the blood stream. Experiments in animals suggest that this may first become prominent when the concentration falls to about 0.1 per cent of the dry weight of the gland or about one half of the normal concentration (94). When the supply of hormone to the tissues decreases, the anterior pituitary gland increases its secretion of thyrotropic hormone. In consequence the thyroid gland becomes hyperplastic, the mass of active tissue increases, the blood supply enlarges, and the ability to accumulate iodide ion against a concentration gradient is enhanced. These compensatory reactions increase the rate at which the gland clears iodide from the blood stream (equation 1) and ultimately enable it to meet successfully the competition of the kidney for the available supply of iodide. The new equilibrium which is established is illustrated in Figure 6. The thyroid gland is now subsisting largely upon the iodide returned to the blood stream by breakdown of hormone in the tissues. Although the renal *clearance* of iodide remains constant, the total *quantity* of iodide lost in the urine is greatly diminished because the concentration of iodide ion in the iodide compartment has fallen to less than 0.2 microgm. per liter (see Table 3).

In Figure 6 it has been assumed for simplicity that complete compensation has been achieved in the face of a diminished iodide intake. However, theoretically compensation must fall somewhat short of its mark else the anterior pituitary would decrease its stimulus and the thyroid gland would tend to revert to normal, as indeed it does if the iodine deficiency is promptly corrected. Presumably the concentration of thyroid hormone perfusing the pituitary must remain somewhat low, yet among a large number of goiterous subjects studied in Argentina only a few had significantly decreased concentrations of protein-bound iodine in the serum (179). Probably also the very large goiters which are often caused by iodine deficiency are due to prolonged, rather than maximal, stimulation by thyrotropic hormone. Further increases in thyroid function can still be produced by the administration of exogenous thyrotropic hormone (179).

Astwood (8) has raised the interesting question of whether a decreased concentration of iodine in the thyroid gland may itself stimulate thyroid activity without the participation of the anterior pituitary. If this mechanism exists, it might of course contribute to the development of goiter, but it hardly seems necessary to invoke any such additional stimulus.

It should be emphasized that the simple cycle of events just described applies only to the initial stage of endemic goiter. Persistent stimulation by endogenous thyrotropic hormone seems to result in man, as it does in animals (114, 115), in the development of more or less discrete thyroid adenomas which are extraordinarily variable in histological structure. It is conceivable that some of these nodules may become autonomous and continue to function independently of thyrotropic stimulation. If the iodine deficiency is corrected, such nodules might feast upon the newly abundant iodide and secrete hormone without regard for bodily needs, even though the support of the anterior pituitary be withdrawn. This would explain the occurrence of "Jodbasedow", a phenomenon whose very existence has been questioned in recent years. Regardless of the mechanism involved, the reviewer feels that the transient increase in the incidence of hyperthyroidism when iodized salt was first introduced in the midwest (73, 84, 102, 103, 135) was not only very real but difficult to explain by anything except the increased supply of iodide.

---

[6] A possible reason for the increasing difficulty is discussed in Section X-B.

The production of goiter by iodine deficiency is no longer hypothetical. Unless compensatory mechanisms fail, long continued ingestion of a diet markedly deficient in iodine inevitably causes enlargement of the thyroid gland, with an increase in blood supply sufficient to allow the daily collection of a normal total quantity of iodide despite a much reduced concentration in the blood stream. The only alternative would be to assume that a gland of normal size could accommodate a liter and a half of blood per minute and could extract the iodide from this torrent with normal efficiency. Since this alternative is obviously ridiculous, the recurring argument that iodine deficiency is not a sufficient cause of endemic goiter (55, 56, 57) is finally and utterly demolished. *In euthyroid persons with normal renal function goiter is an obligatory response to prolonged and severe iodine deficiency.* This categorical statement must not be interpreted to mean that iodine deficiency is the *sole* cause of goiter even in regions where the supply of iodine in food and water is minimal. Any other factor which impairs the efficiency of hormone synthesis or which increases the requirement for thyroid hormone will reinforce the goitrogenic effect of a diminished iodide intake.

G. *Other Dietary Factors:* Astwood and his coworkers have conducted a remarkable series of experiments on the thyroid-inhibiting properties of various foods (61). These investigations led to the isolation of an active goitrogenic agent, l-5-vinyl-2-thioöxazolidone, from various brassicaceous plants (9). Pharmacologically this naturally occurring goitrogen resembles the synthetic drugs of the thioamide series. The influence of diet on thyroid function and iodine metabolism has been competently reviewed by Greer (58).

IX. PATHOLOGICAL VARIATIONS IN IODINE METABOLISM AND THYROID FUNCTION

In this section the discussion will be limited to those types of thyroid disease which produce striking aberrations of iodine metabolism. Certain clinically important diseases of the thyroid such as cretinism in regions of endemic goiter, thyroiditis, and benign or malignant tumors of the thyroid gland will not be considered. Furthermore, the differences between diffuse hyperplasia and toxic adenoma will for the most part be disregarded.

A. *Hypothyroidism:* According to the model presented here, the complete absence of functional thyroid tissue should reduce iodine metabolism to the relatively simple processes of absorption, distribution and excretion. In general this expectation is confirmed by the behavior of radioactive iodine in patients with severe myxedema (18, 175). Because the renal clearance of iodide tends to be reduced in patients with hypothyroidism (Table 1) the excretion of a tracer dose of radioactive iodine in the urine is somewhat delayed. But if urine specimens are accurately collected for three or more days, the total quantity recovered in the urine is usually 100 per cent of the administered dose within the error of measurement (18, 175). However, even in patients with severe myxedema Keating and his collaborators have often been unable to recover more than about 85 per cent of the administered dose (82). The significance of the "missing" iodine is quite obscure. Keating is inclined to ascribe it to retention by extrathyroidal tissues. This explanation is unattractive, since it would demand that a very considerable quantity of iodide be sequestered for a long period of time, either in the tissues as a whole or in specific sites. The rate constant of transfer of iodide from the iodide compartment to the sites of accumulation would have to be much larger than the rate constant of transfer in the reverse direction. At equilibrium, the total quantity of iodide in these putative sites of accumulation would therefore have to be greater than in the iodide compartment proper. Since there is no independent evidence for any such sequestration of iodide in relatively high concentration by extrathyroidal tissues, and since the majority of investigators have been able to account for practically 100 per cent of the administered dose, it seems likely that the "missing" iodide can best be explained by small, but consistently negative, errors in the collection of urine samples and in the measurement of radioactivity.

Keating's group has also demonstrated that, even in patients with myxedema, a small amount of radioactive iodine which is precipitable with zinc and therefore presumably organic appears in the blood stream. In one instance the maximum concentration of precipitable radioactive iodine was practically attained within a few hours of administration of the tracer dose, and then remained constant for approximately one week (104). This might possibly represent extrathyroidal synthesis of thyroxine, a process described by Chaikoff and his collaborators in animals (77, 116). But, since in patients with severe myxedema the concentration of protein-bound iodine in the blood stream as determined chemically is usually very close to zero, it is extremely doubtful that significant quantities of hormone are synthesized in man by extrathyroidal tissues. A more likely alternative is the possibility that even in a patient with no detectable accumulation of radioactivity in the neck, there may remain small bits of functional thyroid. Because of intense stimulation by thyrotropic hormone such remnants of thyroid tissue would be expected to secrete whatever hormone they can manufacture as rapidly as possible. This would account for the prompt but very limited appearance of organic radioactive iodine in the blood stream.

B. *Hyperthyroidism:* The changes in iodine metabolism characteristic of hyperthyroidism are illustrated in Figure 7. The primary derangement is the increased rate at which hormone is synthesized and secreted. In diffuse hyperplasia the increased turnover of hormone usually causes a marked reduction in the concentration of stored hormone, but because of the increase in the size of the gland the total quantity is often reduced only moderately. From a review of the literature, Gutman and his coworkers (68) concluded that the mean quantity of organic iodine in the thyroid gland in patients with untreated exophthalmic goiter was 5,500 microgm. They also demonstrated that after treatment with iodide the mean quantity was increased to 28,200 microgm.

In constructing Figure 7 the following assumptions have been made: 1) That the renal clearance of iodide remains normal in hyperthyroidism. This is probably true (see Table 1). 2) That the volume of distribution of the iodide ion is unchanged. Actually Keating and Albert (80) and Myant and his coworkers (119) have reported that the volume of distribution of iodide in hyperthyroidism is somewhat greater than normal. The cause of this increase is not clear. In part it may be due to the technical difficulties of estimating the volume of distribution of iodide when it is being removed from the blood stream very rapidly. It may also be due in part to the increased ability of the hyperactive thyroid gland to concentrate the iodide ion. However, the conversion of inorganic iodide to organically bound iodine is probably so rapid that even in Graves' disease the total quantity of iodide ion within the thyroid gland is small. 3) That the *proportion* of hormone lost from the body in organic form is the same as in the normal subject. While the information available suggests that the excretion of organic iodine may be greater than normal when the concentration of hormone

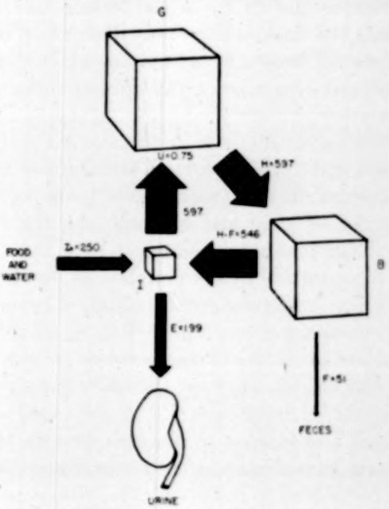

FIG. 7. A diagram of iodine metabolism in a patient with moderately severe thyrotoxicosis (Table 3, column 6). The construction of the diagram has been explained in the legend of Figure 2.

in the blood stream is elevated, there are no reliable measurements of this in man. 4) That the volume of distribution of organic iodine in the tissues is the same as in normal subjects. 5) That because of the increased food consumption characteristic of untreated hyperthyroidism the mean intake of iodide is increased from 150 to 250 microgm. per day.

In Figure 7 the uptake of radioactive iodine is assumed to be 0.75. This is somewhat higher than the mean value for the uptake of radioactive iodine at 24 hours in a large series of patients with hyperthyroidism studied by Keating *et al.* (81). It is somewhat lower than the mean value calculated from the quantity of radioactive iodine excreted in the urine as reported by Skanse (175). The concentration of protein-bound iodine in the plasma has been taken as 14 microgm. per cent. While the data assumed for illustration in Figure 7 are probably typical of moderately severe hyperthyroidism, it should be obvious that the actual values observed in any given patient may differ very widely from the ones chosen for illustration.

A comparison of iodine metabolism in the normal and thyrotoxic subjects illustrated by Figures 2 and 7 reveals certain striking and important differences. In the first place, although the daily secretion of hormone from the hyperactive thyroid gland has increased eight and one-half-fold, the concentration of hormone in the tissue compartment has increased by a little less than three-fold. This implies that the proportion of the hormone in the tissues which is turned over in unit time increases when the concentration of hormone increases. In the examples illustrated, tissue turnover, $K_{BI} + K_{BF}$, is about three times as rapid in the thyrotoxic patient as in the normal subject. A more detailed discussion of the quantitative relationship between the daily supply of hormone and the concentration of hormone in the plasma will be presented in Section XI, A.

In the second place it is of interest to compare the rate of loss of radioactivity from the thyroid gland immediately after accumulation is complete, with the rate of loss when the labeled hormone in the tissues has attained the same specific activity as the hormone in the gland. Solving equation 58 for the rate constant, K_{GB}:

$$K_{GB} = \frac{H}{24Q_G} \tag{58a}$$

Likewise:

$$K_G = \frac{H(1 - U) + UF}{24Q_G} \tag{60a}$$

Dividing equation 58a by equation 60a:

$$\frac{K_{GB}}{K_G} = \frac{H}{H(1 - U) + UF}$$

Solving for K_G:

$$K_G = K_{GB}\left[(1 - U) + \frac{UF}{H}\right] \tag{66}$$

If the values assumed for the normal subject are substituted in equation 66 it is found that the initial rate of loss of radioactive iodine from the gland is only about $1\frac{1}{2}$ times the equilibrium rate of loss. In the thyrotoxic subject, however, the initial rate of loss is more than three times the equilibrium rate. It should be evident from equation 66 that *the true biological half-life of radioactive iodine in the thyroid gland cannot be calculated from the rate of loss of radioactivity immediately after collection is complete* (see Figure 8). Even in the normal subject studied by Burns and his collaborators (21) (see Figure 4) there is perhaps a slight tendency for the radioactivity in the thyroid gland to decrease somewhat more rapidly during the first few days than later on. This tendency is much more clearly evident in curves plotted for several thyrotoxic subjects by Goldsmith and his co-workers (52).

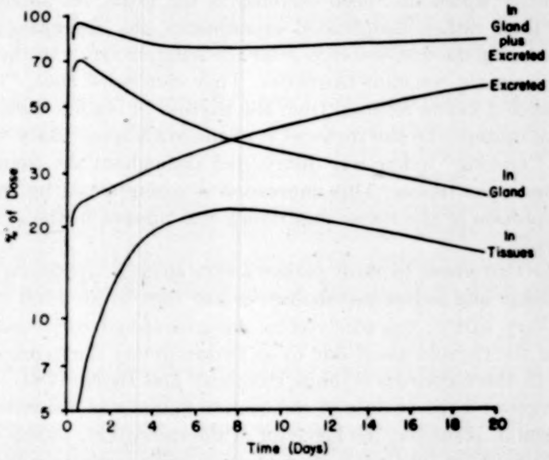

FIG. 8. The theoretical distribution of a tracer dose of radioactive iodine given to the thyrotoxic subject of Figure 7. The curves were not calculated mathematically but were obtained through the kindness of Dr. Gordon Brownell from an electrical analogue computer which he designed and constructed. The scale for per cent of dose is logarithmic.

Note the slow accumulation of radioactive iodine in compartment B, and the changing slope of the curve of decay of radioactive iodine in the thyroid gland. Note also that, as observed by Goldsmith and his collaborators (52), the sum of the radioactive iodine remaining in the thyroid and the radioactive iodine which has been excreted decreases during the first few days after administration of the tracer dose.

In the third place, the rapid turnover of hormone in the gland is likely to result in an underestimate of the true uptake of radioactive iodine, as judged from *in vivo* measurements over the thyroid gland. This is particularly true when the measurements are made at 48 hours after the tracer dose (see Figure 11).

Finally, it should be noted that in thyrotoxicosis the hormone in the plasma and extrathyroidal tissues may constitute a very considerable portion of the total amount of hormone in the body. In the normal subject illustrated in Figure

2, the total quantity of extrathyroidal hormone is only 15 per cent of the quantity in the thyroid itself. In the thyrotoxic subject illustrated in Figure 7, the hormone in the plasma and tissues is 61 per cent of the hormone in the thyroid. In such a patient when the specific activity of the hormone in the tissues has attained the specific activity of hormone in the gland, nearly 40 per cent of the radioactivity remaining in the body would be extrathyroidal (see Figure 8). Goldsmith and his coworkers (52) could not account for all of the radioactivity administered when they summed the quantity remaining in the thyroid gland and the quantity which had been excreted in the urine. As nearly as can be judged from their rather complicated experiments, the discrepancy developed most rapidly during the first few days after the administration of the tracer dose and tended to remain constant thereafter. They concluded that, "15 to 40 per cent of the labeled iodine released from the thyroid in organic form is excreted by alternative routes". To the reviewer it seems much more likely that the majority of the "missing" iodine was distributed throughout the compartment of organic iodine in the tissues. This impression is strengthened by the very considerable proportion of the tracer dose which was present in the serum of some of their patients.

C. *Sporadic Cretinism:* In most patients with sporadic cretinism the thyroid gland is atrophic and iodide metabolism is like that in an adult patient with myxedema. Very rarely, sporadic cretins are enountered with tremendous enlargements of the thyroid gland due to an extraordinary derangement of iodide metabolism. In three goitrous siblings, Stanbury and Hedge (180) found that a large proportion of a tracer dose of radioactive iodine was concentrated within the thyroid gland. However, the behavior of the radioactive iodine was unusual in three respects. First, the peak concentration of radioactivity in the thyroid gland was attained within an hour or two after the oral administration of the tracer. Second, the amount of radioactivity in the thyroid declined very perceptibly during the next few hours. Third, the radioactive iodine was rapidly and almost completely discharged from the thyroid gland by the oral administration of potassium thiocyanate. Simultaneously, the concentration in other parts of the body increased. In two of the glands removed surgically the concentration of protein-bound iodine was only 10.4 and 15 microgm. per hundred grams. In contrast, the inorganic iodide was 29 and 173 microgm. per cent. The larger gland weighed 497 grams and probably contained a total of about 860 microgm. of inorganic iodide.

As the authors point out, the only logical explanation for these observations is that the patients were suffering from a hereditary defect which almost completely prevented the synthesis of thyroid hormone without impairing the ability of the thyroid tissue to maintain a high concentration gradient of inorganic iodide. The cause of the interference with synthesis is unknown, but the end result is remarkably similar to the block produced by drugs of the thioamide group. In essence these glands simply represented an enormous and completely useless extension of the iodide compartment. In the patient with the largest goiter, the iodide content of the thyroid tissue suggests that the volume of distribution of inorganic iodide must have been several times greater than the total volume of the body!

X. THE INFLUENCE OF DRUGS ON IODINE METABOLISM AND THYROID FUNCTION

Since there have been several recent reviews of the mechanism of action of antithyroid compounds, the discussion in this section will be limited to a cursory examination of the effect of certain drugs on iodine metabolism as represented by the simplified model. Dinitrophenol (49, 205), cortisone and adrenocorticotropin (1, 14, 46, 76, 113, 130), and blocking agents of the para-aminobenzene group (8) will not be considered, primarily because their mechanisms of action are poorly understood.

A. *Exogenous Thyroid Hormone:* Suppression of thyroid function by exogenous thyroid hormone has already been mentioned. The primary site of action is presumably the anterior pituitary gland which discontinues its secretion of thyrotropic hormone and reduces the activity of the thyroid gland to the minimum characteristic of hypophysectomy. In rats ablation of the pituitary diminishes the uptake of radioactive iodine (146), the turnover of thyroxine in the thyroid gland (147), and the ability of the thyroid to concentrate iodide ion from the blood stream (197) to about 10 per cent of normal. These observations are consistent with the earlier report of Chaikoff *et al.* (117) that hypophysectomy greatly retards the rate at which diiodotyrosine is converted to thyroxine. In man, Greer's study of the effect of thyroid on the uptake of radioactive iodine in euthyroid subjects also suggests that thyroid activity is very much depressed, but not completely abolished, when the pituitary is inhibited (59). Greer also found that much larger amounts of exogenous thyroid hormone were required to inhibit uptake in patients with hyperthyroidism (60). While a discussion of the role of thyrotropin in thyrotoxicosis is beyond the scope of this paper, the ability of tetrabromthyronine to cause a decrease in the concentration of protein-bound iodine in the serum of patients with Graves' disease suggests that in this type of hyperthyroidism the thyroid gland may indeed be under pituitary control, and may be inhibited by exogenous hormone (92). There are two obvious advantages in using a brominated or chlorinated thyronine rather than thyroxine itself in studies of this sort. First, any inhibitory effects cannot be ascribed to the action of iodide ion derived from breakdown of the exogenous hormone. Second, since the exogenous hormone does not contain iodine, a reduction in the concentration of protein-bound iodine in the serum may be taken as an indication that the production of endogenous hormone has decreased. It is to be hoped that future studies with tetrabromthyronine or tetrachlorthyronine will provide additional quantitative information concerning the inhibitory effect of exogenous hormone in patients with thyrotoxicosis.

Besides inhibiting the secretion of thyrotropic hormone, exogenous hormone may also have a direct inhibitory effect on the thyroid gland itself (33, 151). However, in man this direct effect upon the thyroid is probably rather unimportant (129).

B. *Iodide:* The effects of an increased supply of iodide on the thyroid gland are exceedingly complex and poorly understood. In the *normal* subject the administration of small amounts of iodide such as occur in iodized salt will for a time produce a non-equilibrium state which is precisely the reverse of iodide deficiency. The rate of manufacture of the thyroid hormone exceeds the rate of secretion and the total quantity of hormone stored in the gland therefore increases. Eventually, however, a new equilibrium is attained so that the uptake of iodide again balances the secretion of thyroxine. It is not entirely clear by what mechanism the uptake of iodide is decreased. It would seem logical to suppose that as the concentration of hormone in the colloid rises it becomes easier and easier for the thyroid cells to secrete hormone into the blood stream. De Robertis (42) has suggested that, before being secreted, thyroxine is released from thyroglobulin by a proteolytic enzyme whose activity is controlled by the thyrotropic hormone. If it be assumed that each unit of thyrotropic hormone promotes the proteolysis of a certain quantum of thyroglobulin, the amount of thyroxine thereby made available for secretion would depend upon the concentration of thyroxine in the thyroglobulin. When the concentration increases, the quantity of thyrotropic hormone which formerly just sufficed to stimulate the secretion of the normal daily supply of thyroid hormone now causes the secretion of a slight excess. This would tend to inhibit the secretion of thyrotropic hormone from the pituitary. In consequence, thyroid activity would decrease towards the level characteristic of hypophysectomy. The rate of uptake and turnover of iodine would therefore decline until the concentration of hormone in the blood stream returned to normal.

While this hypothesis offers a satisfactory explanation for the eventual inhibitory effect of a small increase in the daily supply of iodide, it can scarcely explain the achievement of equilibrium when several *grams* of iodide are administered daily. If the effects of hypophysectomy in man are at all comparable to those in the rat, the total withdrawal of hypophysial support should not decrease thyroid activity to less than about 10 per cent of normal. Yet to maintain equilibrium in the face of a continued intake of 1 gram of iodide per day, the uptake of iodide must be reduced to about 0.00007! Somehow the gland effects this all but complete suppression of uptake, and still manages to maintain its normal rate of secretion. It might be postulated that without any thyrotropic stimulation the residual activity of the thyroid gland might still be just sufficient to maintain normal synthesis and secretion of hormone as long as an optimal concentration of iodide was being supplied to the enzyme system concerned with synthesis. Greater increases in iodide supply could not further increase hormone synthesis since the synthetic mechanism would already be working at its maximal capacity. There is at present no acceptable evidence to support this hypothesis. Furthermore, it would imply that hypothyroidism secondary to pituitary failure could be treated by the administration of iodide. This seems unlikely, although so far as the reviewer is aware it has not actually been tried.

The acute effects of large doses of iodide in normal animals and humans have been more extensively studied. Wolff and Chaikoff (202) described a prompt and virtually complete suppression of hormone synthesis. However, the inhibition of organic binding of iodine could not be prolonged beyond about two days, even when high concentrations of iodide were maintained in the plasma (203). In man a similar acute effect of doses of iodide sufficient to raise the plasma concentration to between 6 and 12 microgm. per cent was observed by Stanley (181). However, as Keating *et al.* point out (28), it is impossible solely by *in vivo* observations to prove conclusively that organic binding of iodide has been completely inhibited.

In patients with *Graves' disease* the daily administration of iodide in doses of about 1 mgm. usually produces no obvious change in the severity of the disease. Occasionally, however, such doses may provoke a striking increase in toxicity (190, 192). This suggests that in thyrotoxicosis the rate of secretion of thyroid hormone is sometimes limited by the quantity of iodide available. An increase in the supply of iodide removes this limitation, the manufacture and release of hormone are accelerated, and an exacerbation results.

In contrast, the daily administration of iodide in doses of 6 mgm. or more produces a rapid amelioration of signs and symptoms. In patients with exophthalmic goiter the response is obtained with such regularity that it may be used as a diagnostic test. In patients with toxic nodular goiter, the response to large doses of iodide is much less dramatic and may be entirely absent. The improvement produced by iodide in Graves' disease, though rapid, is usually incomplete. In some patients the improvement may be sustained for long periods if the administration of iodide is continued. In others, the disease may increase in severity even though iodide medication is faithfully maintained. If iodide be withdrawn, a prompt exacerbation is likely to occur, an exacerbation which is sometimes alarmingly severe, probably because the gland has made good its opportunity to store large quantities of thyroid hormone during the administration of iodide (26, 100).

The response to iodide is certainly due to a very rapid decrease in the rate at which hormone is secreted, but the reason for this decrease in secretion remains obscure. As in normal subjects, the acute administration of large doses of iodide to patients with thyrotoxicosis produces a prompt but presumably transient decrease in the organic binding of iodine by the thyroid (181). The blood concentration required for this inhibition is smaller than in the normal subject. Since the hyperplastic thyroid gland is able to maintain a much higher concentration gradient of iodide ion than the normal gland, it has been suggested that the inhibitory effect depends not on the concentration of iodide in the blood stream, but rather on the concentration of iodide within the thyroid gland (140, 181). Childs and his collaborators studied the effects of graded doses of carrier iodide on the accumulation of radioactive iodine (28). A dose of 0.1 mgm. produced no change in uptake. When the dose was progressively increased to 10 mgm. the proportion of the iodine utilized for hormone synthesis gradually decreased. Iodide still accumulated in the thyroid but apparently remained largely in the inorganic form since it tended to decrease rapidly as the kidneys excreted iodide from the body, and could readily be discharged by the administration of thiocyanate. Finally, even the iodide concentrating mechanism was saturated by doses of 500 mgm. or more. These observations show that the hyperplastic thyroid gland may actually accumulate much more iodide ion than it can utilize for the synthesis of hormone. But the decrease in the *proportion* of the available iodide which is used for manufacture of thyroxine cannot explain the iodide response in Graves' disease. Actually, during treatment with iodide the *absolute amount* of hormone stored in the thyroid gland and available for secretion is greatly increased. Further discussion of the confusing and contradictory observations and hypotheses concerning the inhibitory effect of iodide in exophthalmic goiter would serve no useful purpose. An excellent summary of the topic has been presented by Pitt-Rivers (133).

An additional perplexing effect of large doses of iodide is their ability to cause a "spurious" increase in the concentration of protein-bound iodine in the plasma (37, 38, 39). This is apparently due to the formation of a non-calorigenic organic compound of iodine. During prolonged treatment with large doses of iodide the unknown compound accumulates very slowly in the plasma, attains a steady concentration during the course of one or two months, and disappears from the body as slowly as it accumulated when the administration of iodide is discontinued. The substance is probably not formed in the thyroid gland since it appeared in the blood stream in two patients with myxedema (38).

In most body tissues conditions are distinctly unfavorable to the oxidation of iodide and its incorporation into an organic molecule. And even if minute traces of iodide are oxidized in certain tissues, one would expect that, as in the thyroid gland, iodinated derivatives of tyrosine would be formed. However, in the acid secretion of the gastric mucosa small quantities of free iodine might easily occur, and could then iodinate some organic compound present in the gastro-intestinal tract. If this hypothetical precursor were a constituent of bile, the iodinated compound formed from it might well undergo enterohepatic circulation which would account for its slow accumulation in the plasma and its equally slow disappearance.

C. *Thiocyanate and Other Anions:* The ability of the thiocyanate ion to inhibit the concentration of the iodide ion by the thyroid gland has been clearly demonstrated by a number of techniques (183, 198, 204). Wyngaarden and his collaborators (209) have recently shown that this property is not peculiar to thiocyanate but is shared by several other anions. In fact perchlorate was ten times as potent as thiocyanate in its ability to prevent iodide accumulation or to discharge iodide which had already accumulated in the thyroid gland. In many respects these anions behave as though they competed with iodide for whatever mechanism is responsible for maintenance of a concentration gradient of iodide ion across the cell membrane. This mechanism is probably an energy-requiring transfer system, although the presence of an iodide-binding protein in the thyroid gland has been postulated as an alternative mechanism (109, 165, 208). It is noteworthy, however, that, in contrast to iodide, only a very small quantity of thiocyanate ion enters the thyroid gland (206, 207), and that even very large concentrations of thiocyanate do not completely abolish the ability of the thyroid gland to concentrate the iodide ion (140). For further discussion of the possible mode of action of thiocyanate, perchlorate and similar anions, the reader should consult the original papers.

Only a small proportion of patients who are treated for hypertension with thiocyanate develop enlargement of the thyroid gland (10). Furthermore, the goitrogenic action of thiocyanate is rather easily overcome by the simultaneous administration of iodide (6). These observations suggest that the accumulation of a high concentration of iodide ion is not a prerequisite for the manufacture of a normal daily quantity of hormone. When the supply of iodide is generous, the thyroid can obtain the quantity of iodide which it requires even when almost completely deprived of its ability to produce a concentration gradient. However, when the supply of iodide is reduced, the decreased efficiency due to inhibition of the concentrating mechanism becomes a serious handicap. The ultimate consequences are very similar to those of iodine deficiency except that more or less severe hypothyroidism occurs more frequently in patients with goiter due to the administration of thiocyanate.

When the administration of thiocyanate is discontinued, the ability of the thyroid gland to accumulate iodide ion is restored as the thiocyanate is slowly eliminated by the kidneys. As in iodine deficiency (179), the hyperplasia of the thyroid gland decreases rather slowly because of the time needed to reaccumulate an adequate concentration of stored hormone. During recovery the uptake of a tracer dose of radioactive iodine, previously somewhat depressed by the thiocyanate, is often abnormally high (16).

D. *Goitrogenic Drugs of the Thioamide Series:* All of the drugs in this group, which includes thiourea, thiouracil, 2-mercaptoimidazole, and their several derivatives, possess in common an actual or potential —SH group which makes them strong reducing agents and probably accounts for their ability to inhibit the iodination of tyrosine. While there is still doubt concerning their precise mode of action (7, 8, 41, 133), it is well established that adequate doses can produce a practically complete block of thyroid hormone synthesis without in any way altering the ability of the thyroid gland to accumulate iodide ion from the blood stream. The resulting derangement in iodine metabolism is illustrated in Figure 9. In this figure it is assumed that enough blocking agent has been given to the patient of Figure 7 to arrest completely the transfer of iodine from the iodide compartment to the compartment of organic iodine in the thyroid gland, and that this complete block has been maintained for two days. It is also assumed that the thyroid gland has not yet encountered difficulty in mobilizing the large quantity of thyroid hormone necessary to maintain its high rate of secretion. However, it is quite apparent from the figure that if the block of hormone synthesis continues, the delivery of hormone to the blood stream will soon slacken because of approaching exhaustion of hormone previously synthesized and stored in the thyroid. This explains why the response to blocking agents is usually very much more rapid in patients with thyrotoxicosis than in normal subjects. With a complete block, the rapidity of response should theoretically be proportional to K_{GB} which for this subject with thyrotoxicosis is about $12\frac{1}{2}$ times the value for the normal subject illustrated in Figure 2. However, it should be borne in mind that drugs of the thioamide series may actually hasten the rate at which iodine is lost from the thyroid gland (see Section VI, B, 3). This possibility has been disregarded in the example used here.

Another less obvious effect of blocking agents of this type is also illustrated in Figure 9. The size of the iodide compartment has increased more than six-fold. Two factors contribute to this increase. One is a sudden expansion of the volume of distribution of the iodide ion when the synthesis of thyroid hormone is eliminated. In the unblocked gland the rapid removal of iodide ion for the synthesis of thyroid hormone prevents the actual accumulation of any large quantity of iodide within the gland. During the block, removal no longer occurs and the potential "iodide space" within the gland fills to the capacity determined by the concentration gradient which can be maintained. In the hyperplastic thyroid gland of the rat the concentration gradient may be as high as 250 to 1 (198). In Graves' disease the gradient is probably of the same order of magnitude (28, 183). With a gradient of 250 to 1, the iodide space of a 60 gm. thyroid would therefore be equivalent to 15 liters of the volume of distribution of iodide.

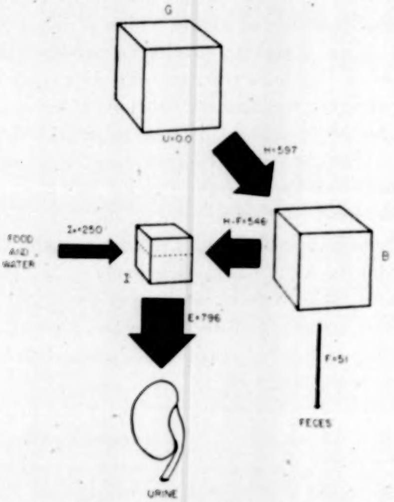

FIG. 9. A diagram of iodine metabolism in the thyrotoxic patient of Figure 7 after two days of complete blockage of thyroid hormone synthesis produced by the administration of a thioamide drug (Table 3, column 7). The construction of the diagram has been explained in the legend of Figure 2.

The portion of the iodide compartment above the dotted line in the figure represents iodide ion within the thyroid gland. The portion below the dotted line represents iodide elsewhere in the body. In addition to the expansion of the iodide compartment, note the enormous increase in the urinary excretion of iodide.

The greater part of the increase in the size of the iodide compartment is not due to the expanded volume of distribution, but rather to the failure of the thyroid gland to remove any of the iodide ion from the iodide compartment. Large quantities of iodide continue to enter the compartment from breakdown of hormone in the tissues, and smaller quantities continue to be absorbed from the gastro-intestinal tract. Their removal now depends solely on clearance by the kidneys. Consequently the concentration of iodide rises until the kidneys are able to excrete iodide as rapidly as it is entering. The increased concentration of iodide is so great that it should be easy to detect by chemical methods.

When the synthesis of thyroid hormone is blocked, the rapid entry of radioactive iodine into the thyroid gland should not be mistaken for a true uptake of iodine. Under these circumstances the accumulation of radioactive iodide does not signify any corresponding net *transfer* of stable iodide but simply indicates that the radioactive isotope is attaining distribution equilibrium with the high concentration of stable iodide already present in the gland. Within an hour or two the iodide compartment will have become uniformly labeled and the peak concentration of radioactive iodine in the thyroid gland will have been reached. Thereafter the concentration of radioactive iodide in the thyroid gland will decrease at the same rate as in other segments of the iodide compartment, the rate of decrease depending on the renal clearance of iodide and the volume of distribution of the iodide ion.

If thiocyanate be administered when a large proportion of the tracer dose has entered the thyroid in the form of iodide ion, the sharp decrease in the volume of distribution of iodide due to inhibition of the concentrating mechanism will rapidly reduce the amount of radioactive iodine in the thyroid to about the same concentration as in other tissues. At the same time, the iodide displaced from the thyroid gland must be accommodated by the remainder of the iodide compartment. In consequence the concentration of both stable and radioactive iodide in the blood stream may rise. This was clearly evident in some of the goitrous cretins in whom the block of synthesis of hormone, though apparently a congenital defect, was very similar to the block produced by drugs of the thioamide group (see Section IX, C).

Maintenance of a complete block of hormone synthesis depends upon maintenance of an adequate concentration of drug within the thyroid gland. In clinical practice it is very probable that a complete block is not usually maintained throughout 24 hours, either because the single doses are not sufficiently large or the interval between doses is too long. The net effect upon iodide metabolism is a greater or lesser reduction in the efficiency of hormone synthesis. The clinical response ultimately achieved and the rapidity with which it is attained will then depend not only on how much the efficiency has been impaired, but also on the concentration of iodide ion in the blood perfusing the gland. Unless a reasonably complete blockage of synthesis is produced, the concomitant administration of iodide may slow the response.

XI. DIAGNOSTIC TESTS

The quantitative relationships discussed earlier will now be used to define the factors which influence certain diagnostic tests. In evaluating such tests it is important to bear in mind that *the clinician is not usually interested in the activity of the thyroid gland, but rather in the action of the thyroid hormone.* Hence the ideal diagnostic test would measure some metabolic effect specifically produced by the hormone in the tissues. Unfortunately no such test is available. While the basal metabolic rate is undoubtedly determined in part by the action of the thyroid hormone, it is also influenced by a wide variety of other factors which are difficult to appraise and impossible to express mathematically. Moreover, the range of variation among euthyroid individuals is very wide. In spite of these well-recognized disadvantages, the basal metabolic rate is still exceedingly useful not only as a diagnostic aid, but also as a means of evaluating the effects of treatment.

The remaining diagnostic tests will be discussed in order of decreasing specificity as measures of the rate of secretion of the thyroid hormone. Although from the theoretical standpoint this will also be the order of decreasing diagnostic value, the practical clinical value of any diagnostic test depends not upon specificity alone but also upon the simplicity and accuracy of the method used. Above all, it depends upon the ability of the test to discriminate between euthyroidism and minimal hyperthyroidism or hypothyroidism. Discriminatory ability can be predicted only in part from a theoretical consideration of the factors which influence a diagnostic test. Clinical utility must finally be established by clinical trial.

A. *The Concentration of Protein-bound Iodine in the Plasma:* The administration of organic compounds of iodine, especially those used as contrast media for roentgenographic diagnosis (97, 148, 154, 169) or the administration of large doses of inorganic iodide (see Section X, B) may cause an elevation of protein-bound iodine in the plasma which bears no relation to thyroid activity. But in the absence of such medication, the concentration of iodine which is bound to the plasma proteins may be taken as a measure of (B), the concentration of hormone in the extrathyroidal compartment. Although the vast majority of the protein-bound material is thyroxine, traces of two other substances have been detected by chromatography (65). One of these has recently been identified as 3:5:3′-l-triiodothyronine by Gross and Pitt-Rivers (66) who also synthesized the compound and showed that it was about three times as potent as l-thyroxine in preventing goiter in rats treated with thiouracil. Because of this surprisingly high potency, Gross and Pitt-Rivers have suggested the possibility that l-triiodothyronine may be the form of the thyroid hormone that is active in the tissues (67).

In Section IX-B it was pointed out that the proportion of the hormone in the tissues which is turned over in unit time probably increases when the concentration of hormone increases. An hypothesis concerning this relationship will now be developed. In so doing, no attempt will be made to distinguish between turnover due to breakdown of hormone in the tissues and turnover due to excretion of hormone. For convenience, therefore, a single rate constant of tissue turnover, K_B, may be defined:

$$K_B = K_{BI} + K_{BF} \qquad 67$$

The simplest possible assumption concerning a relationship between K_B and (B) will now be made, namely that K_B is directly proportional to (B). Hence:

$$K_B = c\ (B) \qquad 68$$

Where c is a proportionality constant with dimensions l^3, m^{-1}, t^{-1}. c may be defined as the liters of compartment B cleared of hormone per hour per microgm. of hormonal iodine present in the compartment. A numerical estimate of c may be obtained by substituting in equation 68 the "typical" values of K_B and (B) given in Table 3 for the normal subject and for the hyperthyroid subject. For the normal subject:

$$0.00222 + 0.00021 = 50\ c$$

$$c = 0.0000486 \text{ liters per hr. per microgm.}$$

For the thyrotoxic subject:

$$0.00676 + 0.00063 = 140\ c$$

$$c = 0.0000528 \text{ liters per hr. per microgm.}$$

The mean of these two estimates of c, 0.0000507, may now be substituted in equation 68:

$$K_B = 0.0000507\ (B) \qquad 68a$$

Combining equations 63 and 64:

$$(B)V_B = \frac{H}{24K_B} \qquad 69$$

Substituting the value for K_B from equation 68a in equation 69:

$$(B)V_B = \frac{H}{0.00122(B)}$$

or

$$(B)^2 = \frac{H}{0.00122V_B} \qquad 70$$

Taking V_B as constant at 24 liters, and substituting this value in equation 70:

$$(B)^2 = 34.1\ H \qquad 71$$

For convenience in testing the validity of equation 71, it may be expressed in logarithmic form:

$$2 \log (B) = \log H + \log 34.1$$

or

$$\log (B) = 0.5 \log H + 0.766 \qquad 72$$

If the assumptions involved in the derivation of equation 72 are reasonable, a plot of log (B) against log H should give a straight line with a slope of 0.5 and an intercept of 0.766.

A truly critical test of the hypothesis just presented would demand simultaneous measurement of the protein-bound iodine and of the daily secretion of hormone in a series of patients with various degrees of hyper- and hypothyroidism. Although methods for estimating the daily secretion of hormone have been developed (see Section VI-B), they have not yet been applied to any such series of patients. However, data concerning the effect of various doses of exogenous hormone on the concentration of protein-bound iodine are available both for hypothyroid patients (201) and for euthyroid subjects (158). These data are presented in Table 4, and plotted in Figure 10. In order to include the data obtained before thyroid was administered, equation 71 has been used to calculate the secretion of endogenous hormone from the observed values of (B). By so doing, the initial point in each series is deliberately placed upon the theoretical line. These two points, therefore, contribute no information about the agreement between observation and hypothesis. However, the positions of the remaining points in both series are practically uninfluenced by the endogenous production of hormone. The striking agreement between these experimental points and the theoretical line tends to support the assumption of a linear relationship between K_B and (B).

TABLE 4

The effect of variations in the daily supply of thyroid hormone on the concentration of protein-bound iodine in serum

SUBJECTS	DOSE OF THYROID	MEAN PROTEIN-BOUND IODINE OF SERUM	DAILY SUPPLY OF THYROXINE IODINE		
			Endogenous* (calculated†)	Exogenous‡	H Exogenous plus endogenous
	mgm/day	*microgm./l.*	*microgm/day*	*microgm./day*	*microgm./day*
8 patients with hypothyroidism (201)	0	11	3.5	0	3.5
	65	36	3.5	35.0	38.5
	97.5	45	3.5	52.5	56.0
	130	54	3.5	70	73.5
4 euthyroid subjects (158)	0	53	82.5	0	82.5
	195	66	0	105	105
	390	77	0	210	210
	650	106	0	350	350
	975	128	0	525	525

* It is assumed that, when as much as 195 mgm. of thyroid is given to a euthyroid subject, endogenous production of hormone ceases (59).

† Calculated from the protein-bound iodine of serum by the use of equation 71.

‡ Assuming that 130 mgm. of thyroid by mouth are equivalent to 70 microgm. of endogenous thyroxine iodine (see Section VI-B).

A further, but less satisfactory, test of this hypothesis depends upon its ability to predict the relationship between the uptake of radioactive iodine and the concentration of protein-bound iodine. Substituting in equation 71 the value for H from equation 15:

$$(B)^2 = 34.1E\left(\frac{U}{1 - U}\right) \qquad 73$$

Skanse (175) has plotted 0.1 (B), the serum protein-bound iodine in microgm. per cent, against 100 (1 − U), the per cent of a tracer dose of radioactive iodine excreted in forty-eight hours, for a large series of patients with hyperthyroidism. Since the excretion of stable iodide was not determined, it is necessary to assume a constant mean value for E which may be taken as approximately 200 (Table 3). Then equation 73 becomes:

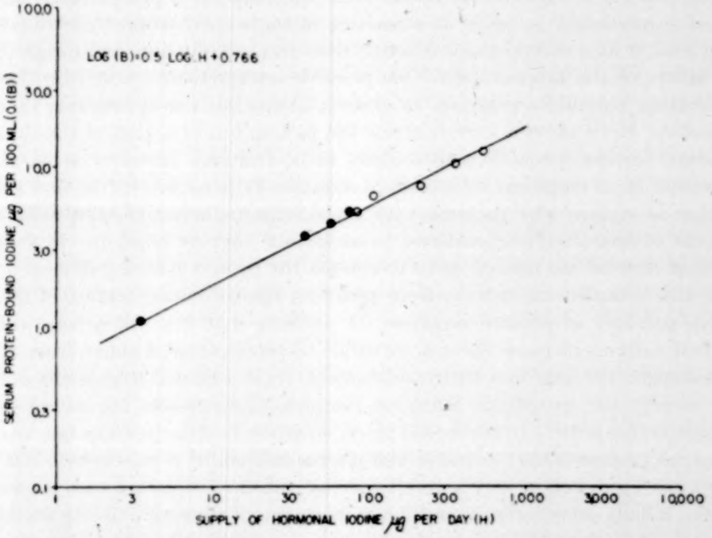

FIG. 10. The relation between the daily supply of thyroid hormone and the serum protein-bound iodine. Both scales are logarithmic. The straight line represents the theoretical relationship defined by equation 72. The calculation of the points is given in Table 4. Solid circles, myxedematous subjects; open circles, euthyroid subjects.

$$(B)^2 = 6820\left(\frac{U}{1 - U}\right) \qquad 73a$$

The line determined by equation 73a describes fairly well the general trend of the points plotted by Skanse, but the observed excretion of radioactive iodine for any given concentration of protein-bound iodine tends to be somewhat lower than the equation predicts. This is to be expected, since in practice the observed excretion of radioactive iodine is apt to be less than the true value of (1 − U) because of incomplete collection of urine.

If equation 73 is used to calculate the values of U corresponding to the normal limits of (B), (35 to 70 microgm. per liter (154)), the predicted normal range of U′ for persons with an iodide excretion of 144 microgm. per day is 0.2 to 0.5. This is reasonably close to the normal limits of 0.17 to 0.58 (175) at 48 hours, and 0.10 to 0.40 (200) or 0.17 to 0.43 (81) at 24 hours which have actually been reported by various investigators.

Equation 71 indicates that the *absolute quantity* of thyroxine which is disposed of each day is a function of the second power of the thyroxine concentration. Consequently, if it be assumed that disposal of thyroxine occurs chiefly by the process of breakdown, rather than excretion, this process may be described, superficially at least, as a reaction of the second order. Unfortunately, classification as a second-order reaction does not provide any real insight into the nature of the process itself. One possible interpretation would be that in accelerating metabolic processes in general, thyroxine also accelerates its own utilization. Until more is known about the mechanism of action of the thyroid hormone, further speculations are likely to be fruitless. However, even as an expression of an empirical relationship, equation 71 is useful. In the first place, it helps to explain why patients with myxedema are sensitive, and euthyroid subjects comparatively insensitive, to exogenous thyroid hormone. If x milligrams of thyroid are needed daily to elevate the protein-bound iodine of a patient with complete myxedema from zero to a normal concentration of 50 microgm. per liter of plasma, equation 71 predicts that 4 x milligrams will be required daily to increase the concentration of protein-bound iodine from 50 to 100 microgm. per liter in a euthyroid subject. (It is assumed that in the euthyroid subject the exogenous hormone completely suppresses the secretion of endogenous hormone.) In the second place, equation 71 indicates why the concentration of protein-bound iodine in the plasma seldom, if ever, exceeds 350 microgm. per liter even in very severe hyperthyroidism. To sustain such a concentration, a daily secretion of about 3,600 microgm. of thyroxine iodine would be required. It is scarcely conceivable that the thyroid gland of any patient with a normal intake of iodide could manufacture and secrete hormone more rapidly than this (see Section XI-D). Finally, it is evident from equation 71 why patients with severe hypothyroidism and no detectable accumulation of radioactive iodine in the thyroid region may still have appreciable quantities of protein-bound iodine in the blood stream (see Section IX-A). According to the equation, about one twenty-fifth of the normal rate of secretion of hormone would maintain a concentration of 10 microgm. of protein-bound iodine per liter of plasma, and one one-hundredth of the normal rate of secretion would maintain a concentration of 5 microgm. per liter. These rates of hormone secretion would require uptakes of only about 2 per cent and 0.5 per cent, respectively.

According to equation 70, the concentration of protein-bound iodine in the plasma should depend only upon the rate of secretion of thyroid hormone and the volume of distribution of the hormone in the extrathyroidal tissues. Presumably the volume of distribution varies within a relatively narrow range, more or less in proportion to body weight. Hence the plasma protein-bound iodine may usually be considered a fairly specific index of the rate at which thyroid hormone is secreted by the gland and utilized by the tissues. However, equation 70 is undoubtedly an oversimplification. In the first place, the constant term, 24 c, is probably not truly constant under all conditions. For example, it may increase during adolescence and decrease in old age (Section VIII-B). It may also increase during exposure to cold, at least in certain species (Section VIII-D). In the second place, in nephrosis the concentration of protein-bound iodine in the *plasma* is low despite compelling evidence that the activity of the thyroid gland is normal or even slightly greater than normal. Recant and Riggs (153) have advanced the hypothesis that a very small quantity of free thyroxine exists in equilibrium with the protein-bound thyroxine of plasma and that it is this minute concentration of unbound hormone which determines the rate of entry of thyroxine into cells. If this theory is correct, a decrease in the thyroxine-binding protein of the plasma, now thought to be α_1 globulin (53), would decrease the concentration of protein-bound iodine needed for maintenance of a *normal* concentration of active free thyroxine in the plasma. Consequently the concentration of hormone *within the cells* in nephrosis would also be normal. If so, the relationship between (B) and H postulated in equation 70 would still be fundamentally correct, except that the ratio of hormone concentration in the plasma to hormone concentration in the cells would be altered, and the plasma protein-bound iodine would therefore not be the usual measure of (B) throughout the whole tissue compartment. This difficulty could presumably be avoided if it were possible to express (B) as the concentration of unbound thyroxine, rather than protein-bound thyroxine, in plasma.

The changes in protein-bound iodine which occur in health and disease have been exhaustively reviewed by Rapport and Curtis (148).

B. *The Uptake of Radioactive Iodine by the Thyroid Gland:* The *theoretical uptake* of radioactive iodine may be defined as the proportion of a tracer dose which would be accumulated by the thyroid in infinite time if there were no secretion of labeled hormone from the gland. The theoretical uptake, herein designated by the symbol U, is a fundamental parameter of thyroid function since it denotes the proportion of the iodide supply which is available for hormone synthesis. In practice, however, it is impossible to measure the theoretical uptake. Some loss of labeled hormone occurs before collection of the administered radioactive iodide is complete, so that the proportion of a tracer dose actually present in the gland at any time after administration is always less than the theoretical uptake. The magnitude of the discrepancy will depend chiefly upon K_{GB}, the ratio of H/24 and Q_G. If serial counts over the gland are available, a fair approximation of the theoretical uptake may be obtained by extrapolating the curve of biological decay back to time zero. The value so obtained may be termed the *extrapolated uptake*.

When the uptake is to be estimated from a single determination of radioactivity in the thyroid, the ideal time for measurement would be the time of *maximum uptake*. However, the time after a tracer dose at which the quantity of radioactive iodine in the thyroid reaches a peak is not constant. In general it will be earlier when K_{GB} is large. Actual measurement of the maximum uptake will therefore also require a series of observations.

The estimate of uptake most commonly used as a diagnostic test may be designated the *fixed-interval uptake*. A single measurement of radioactivity in the thyroid gland is made at an arbitrarily fixed interval of time, usually 24 or 48 hours, after administration of the tracer dose. Although the 48-hour uptake may be appropriate when K_{GB} is small, it will seriously underestimate the theoretical uptake when turnover of hormone in the thyroid gland is brisk. Indeed, when K_{GB} is very large the radioactivity in the thyroid may already have started to decline at 24 hours. It is evident that if uptake is to be estimated from a single measurement at a fixed interval of time, a compromise must be reached between an interval so short that collection by a sluggish gland will be grossly incomplete, and an interval so long that a considerable quantity of labeled hormone will have been secreted by an active gland.

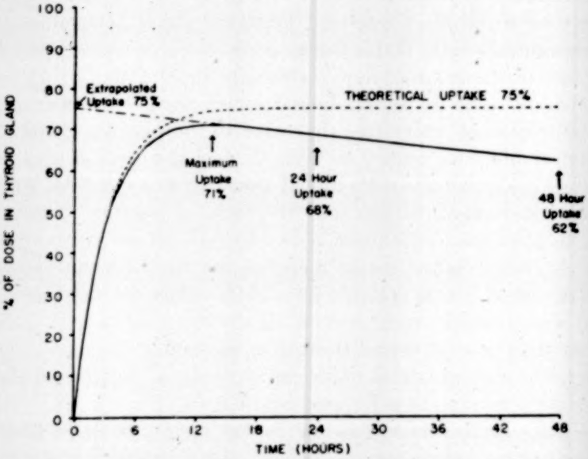

FIG. 11. The relationship between various estimates of the uptake of radioactive iodine. The solid line represents the theoretical curve for the uptake and early decay of radioactive iodine in the thyroid gland after intravenous administration of a tracer dose at time zero to the thyrotoxic subject of Figure 7. The broken line represents the manner in which radioactive iodine would accumulate were there no loss of labeled hormone from the gland. The line of long and short dashes represents an extrapolation of the decay curve to time zero. The various measures of uptake illustrated in this figure are discussed in the text.

The relationship between these various estimates of uptake and the theoretical uptake for the hyperthyroid patient of Figure 7 and Table 3 is illustrated in Figure 11.

Not illustrated is still another method of estimating the uptake, namely, measurement of the proportion of a tracer dose excreted in the urine. Subtraction of this proportion from unity gives the *uptake by difference*. Theoretically, this method has much in its favor since radioactive iodine can be measured more accurately in urine than in the thyroid gland. Furthermore, even if a considerable quantity of labeled hormone is secreted, comparatively little of it will be metabolized to iodide during the period of urine collection (usually 48 hours). But in practice, this method is apt to overestimate the theoretical uptake because of failure to obtain complete collections of urine. The uptake by difference will also be unduly high if the clearance of iodide by the kidneys is much reduced.

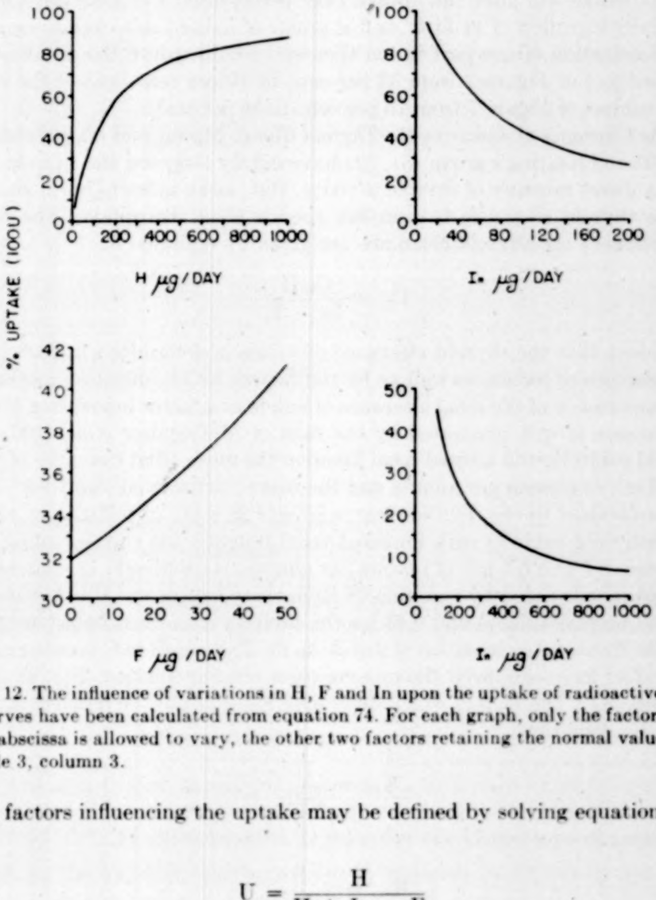

FIG. 12. The influence of variations in H, F and In upon the uptake of radioactive iodine. The curves have been calculated from equation 74. For each graph, only the factor plotted on the abscissa is allowed to vary, the other two factors retaining the normal values listed in Table 3, column 3.

The factors influencing the uptake may be defined by solving equation 13 for U:

$$U = \frac{H}{H + In - F} \qquad 74$$

The uptake is therefore not solely dependent upon the rate of secretion of the thyroid hormone but is also profoundly influenced by the intake of iodide and by the excretion of organic iodine. However, unlike the diagnostic tests to be discussed subsequently, the uptake is unaffected by the renal clearance of iodide. In Figure 12 are illustrated the changes in uptake which are produced when H, In and F are allowed to vary over ranges which may actually be encountered. It is well established that a reduction in iodide intake will eventually lead to an increase in uptake (Section VIII-F) and that the administration of large doses of iodide will practically abolish the uptake (Section X-B). However, it is apparently not widely appreciated that a modest increase in the daily supply of iodide will alter the uptake very perceptibly. For example, the continued daily ingestion of as little as five grams of iodized salt containing iodide in a concentration of one part in ten thousand would reduce the uptake of the normal subject of Figure 2 from 33 per cent to 10 per cent, and of the hyperthyroid subject of Figure 7 from 75 per cent to 46 per cent.

C. *The Clearance of Iodide by the Thyroid Gland:* Myant and his collaborators (121, 123) and Keating's group (81, 82) have rightly proposed the thyroid clearance as a direct measure of thyroid activity. But, as an index of hormone secretion, the thyroid clearance is even less specific than the uptake. The factors which influence the thyroid clearance are given by equation 6:

$$C_G = \frac{C_K H}{In - F} \qquad 6$$

It is evident that the thyroid clearance of iodide is determined in part by the renal clearance of iodide, as well as by the factors which influence uptake.

The importance of the renal clearance of iodide as a factor influencing the thyroid clearance is well illustrated by the data of McConahey *et al.* (105). In 9 euthyroid subjects with normal renal function the mean renal clearance of iodide was 33.3 ml. of plasma per minute and the mean "extra-renal clearance" (practically equivalent to the thyroid clearance) was 26.3 ml. of plasma per minute. In 12 euthyroid subjects with impaired renal function the corresponding mean values were 8.7 and 6.5 ml. of plasma per minute, respectively. In contrast, the mean *uptake* calculated by equation 38 from these figures was 0.44 for the subjects with normal kidneys and 0.43 for the subjects with renal impairment.

D. *The Rate of Accumulation of Iodide by the Thyroid Gland:* Keating and his collaborators have employed the rate constant of accumulation, K_{IG}, as a diagnostic test (82). The factors which determine K_{IG} will now be derived. At equilibrium, by definition:

$$K_{IG} = \frac{H}{24Q_I} \qquad 75$$

Substituting in equation 75 the value for Q_I from equation 44,

$$K_{IG} = \frac{H}{24V_I(I)} \qquad 76$$

Substituting in equation 76 the value for (I) from equation 5a

$$K_{IG} = \frac{0.06C_K H}{V_I(In - F)} \qquad 77$$

K_{IG} is therefore slightly less specific than C_G as a measure of H, since it is influenced by the volume of distribution of iodide as well as by the renal clearance, the intake and the excretion of organic iodine.

If C_K, V_I, In and F remain essentially constant, both the thyroid clearance and the accumulation rate have one theoretical advantage over the uptake. They are *directly* proportional to H and consequently undergo large changes throughout the hyperthyroid range. But in equation 74 for the uptake, **H** appears in both numerator and denominator. As H increases, therefore, the rate of change of U becomes smaller and smaller. For example, if H increases from 150 microgm. per day to 300 microgm. per day (corresponding roughly to a change from high normal to distinctly hyperthyroid), U would only increase from 0.51 to 0.68. In contrast C_G would increase from 35 ml. per minute to 70 ml. per minute, and K_{IG} from 0.083 per hour to 0.167 per hour.

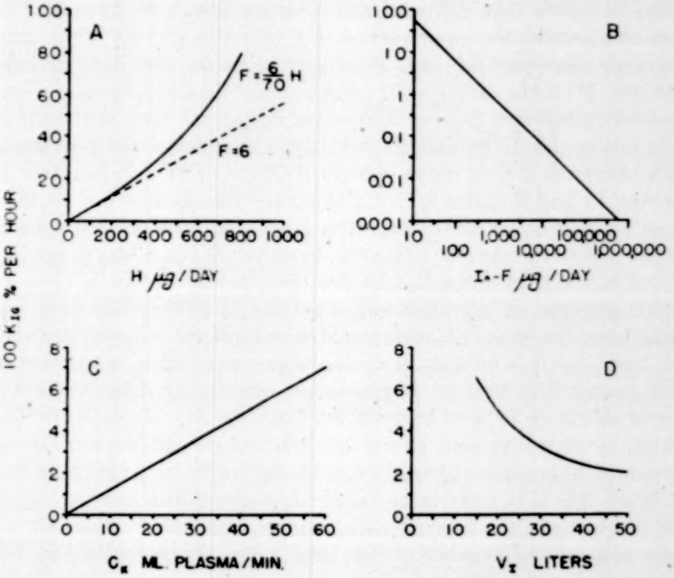

FIG. 13. The influence of variations in H, In-F, C_K and V_I upon K_{IG}, the rate constant of iodine uptake by the thyroid gland, expressed as per cent of the quantity of iodide present in compartment I which is accumulated per hour. The curves have been calculated from equation 77. Except for the solid line in graph A, only the factor plotted on the abscissa is allowed to vary in a given graph. The other factors retain the normal values listed in Table 3, column 3. In the solid curve of graph A, F is taken as 0.0857 H. In curves A, C and D, both scales are arithmetic. In curve B, both scales are logarithmic. Curves A, B and C may also be used to illustrate the influence of the various factors on C_G, the thyroid plasma clearance of iodide, provided that the numerals on the ordinate are multiplied by 4.16. For example, in curve C when the renal clearance is 10 ml. of plasma per minute the rate of accumulation is 1.17 per cent per hour and the thyroid clearance would therefore be 4.85 ml. of plasma per minute.

In Figure 13 are illustrated the changes in K_{IG}, expressed as per cent per hour, which would be produced by variations in H, In-F, C_K and V_I. The first three curves will serve equally well for the thyroid clearance provided the ordinates are multiplied by 4.16 to convert them to a scale of ml. of plasma per minute.

A point of considerable importance is illustrated by Figure 13, part A. The dotted line indicates that if F remained constant despite an increasing rate of secretion of hormone, the accumulation rate would be directly proportional to H. It is much more probable that F actually increases with H as indicated by the solid line. If so, the excretion of organic iodine may well become a very important limiting factor as the rate of secretion of hormone rises. While an increase in iodide intake due to polyphagia may for a time offset the increase in the excretion of organic iodine, it can scarcely continue to do so indefinitely. As the gap between In and F narrows, In-F rapidly becomes smaller and smaller, and K_{IG} must become larger and larger. The same argument obviously applies to the uptake and to the thyroid clearance. In section XI-A it was suggested that the rate of secretion of hormone in hyperthyroidism is seldom, if ever, greater than 3,600 microgm. of thyroxine iodine per day. If 6/70 of this were excreted in organic form, the intake of iodide would obviously have to exceed 309 microgm. per day. And since it is unlikely that the clearance of iodide by the thyroid can be much greater than 1000 ml. of plasma per minute, the daily intake of iodide would probably have to be at least 429 microgm. per day, almost three times the normal intake. This argument clearly implies that in patients with severe hyperthyroidism, improvement would be produced by limiting the daily intake of iodide. While this is of theoretical, rather than therapeutic interest, it is quite possible that some of the "spontaneous" exacerbations and remissions so characteristic of untreated hyperthyroidism may be due to unrecognized fluctuations in the daily intake of iodide.

Astwood (182) has developed a purely empirical measure of accumulation which he has termed the *accumulation gradient*. It may be defined as the slope of the straight line which is usually obtained when the radioactivity in the thyroid gland, expressed as counts per second, is plotted on the ordinate against the square root of the time in minutes on the abscissa. When so defined, the numerical value of the accumulation gradient depends not only upon the rate of accumulation but also upon the dose of radioactive iodine and the method of counting. It would seem preferable to plot the radioactivity in per cent of the administered dose (see Figure 14) so that values from different laboratories could be compared. However, this would not obviate the fundamental objection, clearly recognized by Astwood himself (141), that the accumulation gradient does not measure any parameter of thyroid function which can be defined mathematically. It is therefore impossible to derive any equation which will express the factors influencing the accumulation gradient. Indeed, the relationship between radioactivity in the gland and square root of time is at best only approximately linear, and then only for a variable portion of the curve of accumulation (Figure 14). However, Astwood points out that in spite of its limitations the accumulation gradient is a convenient means of estimating, from a few early observations, the general trend of accumulation, and he has used it to good advantage in studies of the potency and duration of action of antithyroid drugs (182).

E. *The Conversion Ratio:* Several investigators have used diagnostic tests which depend upon the rate at which labeled hormone appears in the blood stream after a tracer dose of radioactive iodine (29, 48, 72, 171, 172, 173). Usually a sample of blood is collected at some fixed interval of time after the administration of the tracer and the total radioactivity in the plasma is measured. The plasma proteins are then precipitated and the radioactivity in the precipitate is measured as an index of the concentration of labeled hormone. The proportion of the total radioactivity which is protein-bound is termed the conversion ratio, C.R. Hence, by definition:

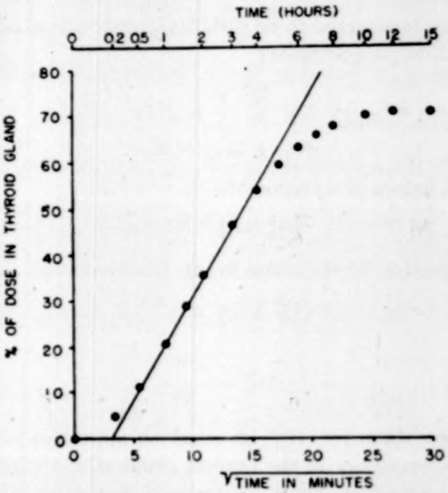

FIG. 14. The theoretical accumulation of radioactive iodine by the thyroid gland of the thyrotoxic subject of Figure 7 (solid circles) plotted against the square root of the time in minutes. For comparison, the time in hours is given on the upper scale.

When plotted in this manner, the curve of uptake is a skewed sigmoid curve. The straight line, whose slope is proportional to the accumulation gradient, fits a portion of the points reasonably well but it is evident from the figure that the exact slope of the line will depend upon which of the points the investigator arbitrarily chooses to disregard.

$$\text{C.R.} = \frac{(\text{B})^{\bullet}_{t}}{(\text{I})^{\bullet}_{t} + (\text{B})^{\bullet}_{t}} \qquad 78$$

A rigorous mathematical analysis of the factors affecting the conversion ratio would require simultaneous solution of equations 41, 42 and 43. The difficulties inherent in this have already been mentioned (Section VII). However, the interval between administration of the radioactive iodine and determination of the conversion ratio is usually only 24 hours, and it is probably safe to assume that during this time very little radioactive iodide derived from breakdown of labeled hormone in the tissues is entering the iodide compartment. This simplifying assumption makes it possible to derive a reasonably accurate expression for the factors which influence the conversion ratio.

$(I)^*_t$, the concentration of radioactive iodide at any time, t, after the administration of a tracer dose may be calculated from equation 46:

$$Q^*_{I_t} = Q^*_{I_0}\, e^{-(K_{IE} + K_{IG})t} \qquad 46$$

But since the tracer is assumed to be instantaneously absorbed and distributed throughout the iodide compartment:

$$Q^*_{I_0} = 1 \qquad 79$$

Also, for convenience, define:

$$K_{IE} + K_{IG} = K_I \qquad 80$$

Substituting these values in equation 46:

$$Q^*_{I_t} = e^{-K_I t} \qquad 81$$

Substituting in equation 81 the value for Q_I from equation 44:

$$(I)^*_t\, V_I = e^{-K_I t} \qquad 82$$

Solving for $(I)^*_t$:

$$(I)^*_t = \frac{e^{-K_I t}}{V_I} \qquad 83$$

To obtain an expression for $(B)^*_t$, an equation must first be derived for $Q^*_{G_t}$, the quantity of radioactivity in the thyroid gland at any time, t, after the administration of a tracer dose. Since it is assumed that within the period now under consideration all of the radioactivity is either in compartments I and G or has been excreted in the urine:

$$Q^*_{I_t} + Q^*_{G_t} + Q^*_{E_t} = 1$$

or

$$Q^*_{I_t} = 1 - Q^*_{G_t} - Q^*_{E_t} \qquad 84$$

Where $Q^*_{E_t}$ is the quantity which has been excreted from time zero to time t. By definition:

$$U = \frac{Q^*_{G_t}}{Q^*_{G_t} + Q^*_{E_t}} \qquad 85$$

Solving for $Q^*_{E_t}$

$$Q^*_{E_t} = Q^*_{G_t}\left(\frac{1}{U} - 1\right) \qquad 86$$

Substituting this value for $Q^*_{E_t}$ in equation 84:

$$Q^*_{I_t} = 1 - Q^*_{G_t} - \frac{Q^*_{G_t}}{U} + Q^*_{G_t} = 1 - \frac{Q^*_{G_t}}{U} \qquad 87$$

Solving for Q^*_{Gt}

$$Q^*_{Gt} = U\,(1 - Q^*_{It}) \qquad 88$$

Substituting in equation 88 the value for Q^*_{It} from equation 81:

$$Q^*_{Gt} = U\,(1 - e^{-K_I t}) \qquad 89$$

Since the assumption is being made that no breakdown or excretion of radioactive hormone occurs during the period under consideration, equation 43 reduces to:

$$\frac{dQ^*_B}{dt} = K_{GB}\,Q^*_G \qquad 43a$$

Integrating:

$$\int_0^{Q^*_{Bt}} dQ^*_B = \int_0^t Q^*_{G_t}\,K_{GB}\,dt \qquad 90$$

Substituting in equation 90, the value for Q^*_{Gt} from equation 89:

$$\int_0^{Q^*_{Bt}} dQ^*_B = \int_0^t U(1 - e^{-K_I t})K_{GB}\,dt \qquad 91$$

Solving for Q^*_{Bt}:

$$Q^*_{B_t} = UK_{GB}\,t + UK_{GB}\left(\frac{1}{K_I}\right)(e^{-K_I t} - e^{-K_I 0})$$

or

$$Q^*_{B_t} = UK_{GB}\left[t + \frac{1}{K_I}\,(e^{-K_I t} - 1)\right]$$

or,

$$Q^*_{B_t} = UK_{GB}\left[t - \frac{1}{K_I}\,(1 - e^{-K_I t})\right] \qquad 92$$

Substituting in equation 92, the value of Q^*_{Bt} from equation 63 and solving for $(B)^*_t$:

$$(B)^*_t\,V_B = UK_{GB}\left[t - \frac{1}{K_I}\,(1 - e^{-K_I t})\right]$$

$$(B)^*_t = \frac{UK_{GB}\left[t - \frac{1}{K_I}\,(1 - e^{-K_I t})\right]}{V_B} \qquad 93$$

The value of $(B)^*_t$ from equation 93 and the value of $(I)^*_t$ from equation 83 may now be substituted in equation 78:

$$\text{C.R.} = \frac{\dfrac{UK_{GB}}{V_B}\left[t - \dfrac{1}{K_I}\,(1 - e^{-K_I t})\right]}{\dfrac{e^{-K_I t}}{V_I} + \dfrac{UK_{GB}}{V_B}\left[t - \dfrac{1}{K_I}\,(1 - e^{-K_I t})\right]} \qquad 94$$

However, equation 94 still contains several terms which are not primary factors. These must now be eliminated. Solving equation 58 for K_{GB}:

$$K_{GB} = \frac{H}{24Q_G} \tag{58a}$$

Multiplying equation 58a by equation 74:

$$UK_{GB} = \frac{H^2}{24Q_G(H + In - F)} \tag{95}$$

Solving equation 9 for K_{IE}

$$K_{IE} = \frac{0.06C_K}{V_I} \tag{96}$$

Substituting this value for K_{IE}, and the value for K_{IG} from equation 77 in equation 80:

$$K_I = \frac{0.06C_K}{V_I} + \frac{0.06C_K}{V_I}\left(\frac{H}{In - F}\right)$$

or

$$K_I = \frac{0.06C_K}{V_I}\left(1 + \frac{H}{In - F}\right) \tag{97}$$

Substituting in equation 94 the value for UK_{GB} from equation 95, and the value for K_I from equation 97:

$$C.R. = \frac{\left(\frac{H^2}{24Q_G V_B(H + In - F)}\right)\left[t - \frac{1 - e^{-\frac{0.06C_K t}{V_I}\left(1 + \frac{H}{In-F}\right)}}{\frac{0.06C_K}{V_I}\left(1 + \frac{H}{In - F}\right)}\right]}{\left[\frac{e^{-\frac{0.06C_K t}{V_I}\left(1 + \frac{H}{In-F}\right)}}{V_I}\right] + \left(\frac{H^2}{24Q_G V_B(H + In - F)}\right)\left[t - \frac{1 - e^{-\frac{0.06C_K t}{V_I}\left(1 + \frac{H}{In-F}\right)}}{\frac{0.06C_K}{V_I}\left(1 + \frac{H}{In - F}\right)}\right]} \tag{98}$$

Even from this approximate equation it is obvious that the conversion ratio is an exceedingly complex function, dependent upon no fewer than eight variables: H, Q_G, V_I, V_B, In, F, C_K and t. Indeed at first glance it might seem quite useless as a diagnostic test. Yet an analysis of how its component parts influence the conversion ratio demonstrates that under normal circumstances it may possess great discriminatory power. The numerator, representing $(B)^{*}_{t}$, consists of two main parts. The part enclosed in parenthesis *increases* very rapidly as the rate of secretion of thyroid hormone increases because it is strongly influenced by its numerator, H^2. The part enclosed in brackets also increases with H, but very much more slowly. At the same time the term in the denominator which represents $(I)^{*}_{t}$ *decreases* very rapidly because, as H increases, the negative exponent of e becomes larger. The net result is that the conversion ratio is (theoretically) almost zero when H is low, rises very rapidly while H is increasing over a small range, and very quickly approaches its maximum value of 1. In Figure 15 the conversion ratio calculated from equation 98 has been plotted against the plasma protein-bound iodine, 0.1 (B), as calculated from H using equation 71. The values for the other variables are those listed for the normal subject in Table 3, and they have been taken as constant throughout. The time is 24 hours. Note that the conversion ratio describes a very steep sigmoid curve as the concentration of protein-bound iodine in the plasma rises from 4.0 to 12.0 microgm. per cent. Its mid-point, the point of maximum discriminatory power, is close to 8.0 microgm. per cent which is usually considered the upper limit of normal. This fortunate circumstance means that, so long as the variables other than H do not differ much from the ones chosen for illustration, the conversion ratio calculated at 24 hours should theoretically distinguish rather sharply between euthyroidism and hyperthyroidism. At the same time it would be useless for the diagnosis of hypothyroidism. Since the sigmoid curve will be shifted to the left if the time interval is increased, a longer time interval might be appropriate for hypothyroidism but would probably be useless for the diagnosis of hyperthyroidism.

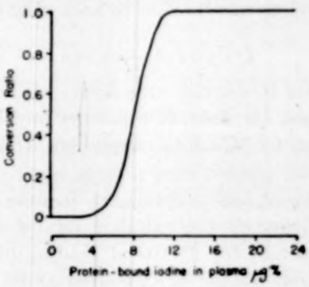

FIG. 15. The conversion ratio, calculated from equation 98 for various values of H, plotted against the concentration of protein-bound iodine in the plasma, calculated from equation 71 for the same values of H. The factors other than H which influence the conversion ratio retain the normal values listed in Table 3, column 3.

Unfortunately, one can never be sure that in a given patient the values of such factors as Q_G, In-F and C_K are actually close to the ones chosen for illustration. Furthermore, accurate measurement of $(B)_t^*$ and $(I)_t^*$ requires the administration of a dose of radioactive iodine too large to be employed legitimately as a tracer for routine diagnostic studies. At present, therefore, the conversion ratio must be interpreted with considerable caution, and does not seem likely to displace the simpler diagnostic tests in clinical practice.

XII. CONCLUSION

It is perhaps appropriate that this review has ended with a consideration of the conversion ratio. *Observation* of the conversion ratio requires only the simplest chemical preparation of a sample of plasma, followed by two determinations of radioactivity. Its *interpretation* has required a rather complicated mathematical analysis, and even this has had to be based upon certain simplifying assumptions not fully in accord with biological facts. It is easy for observation to outdistance precise interpretation.

Yet there is reason to be satisfied with the progress which has already been made towards an orderly arrangement of the observed facts into a solid foundation of quantitative theory. With so much to build upon, further progress should be rapid. In the reviewer's opinion, future advances are likely to stem chiefly from the simultaneous use of several techniques in the detailed investigation of small groups of patients. In particular, he would urge that studies with radioactive iodine be supplemented by the determination of stable iodine not only in blood but also in urine. Many of the present uncertainties concerning quantitative aspects of iodine metabolism in man might thereby be resolved.

ACKNOWLEDGMENTS

Much of the stimulus for this review was derived from the author's many discussions with his colleagues, Dr. John B. Stanbury and Dr. Gordon L. Brownell, during a recent expedition to Mendoza, Argentina. Under Dr. Stanbury's able leadership, the expedition accumulated a large body of quantitative information about the physiology of iodine deficiency in man. The author is particularly indebted to Dr. Brownell for valuable advice concerning some of the mathematical derivations and for a critical reading of the manuscript. He is also indebted to Professors Arthur K. Solomon and Charles F. Mosteller for mathematical advice, and to Dr. F. Raymond Keating, Jr. of the Mayo Clinic, Professor William A. Fish of Providence College and, and Professor A. Stone Freedberg for permission to use unpublished data. The author wishes to acknowledge the secretarial help of Mrs. Beverley W. McClure. Finally he is grateful for the interest and encouragement of Professor Otto Krayer throughout the preparation of this review.

REFERENCES

1. Albert, A.: Thyroid gland. Ann. Rev. Physiol., **14:** 481–498, 1952.
2. Albert, A. and Keating, F. R., Jr.: Metabolic studies with I^{131} labeled thyroid compounds. Comparison of the distribution and fate of radioactive d-l-thyroxine after oral and intravenous administration in the human. J. Clin. Endocrinol., **9:** 1406–1421, 1949.
3. Albert, A. and Keating, F. R., Jr.: Metabolic studies with I^{131} labeled thyroid compounds: distribution and excretion of radiodiiodotyrosine in human beings. J. Clin. Endocrinol., **11:** 996–1011, 1951.
4. Albert, A., Rawson, R. W., Riddell, C., Merrill, P. and Lennon, B.: In vivo augmentation of thyrotropic hormone and partial reactivation of iodinated (inactive) thyrotropic hormone extract by goitrogens. Endocrinology, **40:** 361–369, 1947.
5. Allen, H. C., Jr., Libby, R. L. and Cassen, B.: The scintillation counter in clinical studies of human thyroid physiology using I^{131}. J. Clin. Endocrinol., **11:** 492–511, 1951.
6. Astwood, E. B.: The chemical nature of compounds which inhibit the function of the thyroid gland. J. Pharmacol. & Exper. Therap., **78:** 79–89, 1943.
7. Astwood, E. B.: Chemotherapy of hyperthyroidism. Harvey Lect., **40:** 195–235, 1944–45.
8. Astwood, E. B.: Mechanisms of action of various antithyroid compounds. Ann. New York Acad. Sc., **50:** 419–443, 1949.
9. Astwood, E. B., Greer, M. A. and Ettlinger, M. G.: The antithyroid factor of yellow turnip. Science, **109:** 631, 1949.
10. Barker, M. H., Lindberg, H. A. and Wald, M. H.: Further experiences with thiocyanates; clinical and experimental observations. J. Am. M. A., **117:** 1591–1594, 1941.

11. Barker, S. B.: Determination of protein-bound iodine. J. Biol. Chem., **173**: 715–724, 1948.
12. Barker, S. B.: Mechanism of action of the thyroid hormone. Physiol. Rev., **31**: 205–243, 1951.
13. Berkson, J., Keating, F. R., Jr., Power, M. H. and McConahey, W. M.: Determination of renal clearance of radioiodine. J. Applied Physiol., **2**: 522–529, 1950.
14. Berson, S. A. and Yalow, R. S.: Effect of cortisone on the iodine accumulating function of the thyroid gland in euthyroid subjects. J. Clin. Endocrinol., **12**: 407–422, 1952.
15. Best, C. H. and Taylor, N. B.: The physiological basis of medical practice. 5th Edition, Williams & Wilkins Co., Baltimore, Maryland, 1950.
16. Blackburn, C. M., Keating, F. R., Jr., and Haines, S. F.: Radioiodine tracer studies in thiocyanate myxedema. J. Clin. Endocrinol., **11**: 1503–1511, 1951.
17. Bogoroch, R. and Timiras, P.: The response of the thyroid gland of the rat to severe stress. Endocrinology, **49**: 548–556, 1951.
18. Brownell, G. L.: Analysis of techniques for the determination of thyroid function with radioiodine. J. Clin. Endocrinol , **11**: 1095–1105, 1951.
19. Brownell, G. B. and Stanbury, J. B.: Instrumentation for thyroid measurement. To be published.
20. Brues, A. M.: Biological hazards and toxicity of radioactive isotopes. J. Clin. Investigation, **28**: 1286–1296, 1949.
21. Burns, F. J., Fish, W. A., Hackett, J. W. and Hickey, F. C.: Radioactive decay and metabolic loss of iodine from normal thyroid. J. Applied Physiol., **4**: 15–20, 1951.
22. Carr, E. A., Graham, D. E., Ober, S. and Riggs, D. S.: Catalytic effect of the chromic ion in the Barker method for protein-bound iodine determination. Science, **111**: 552, 1950.
23. Carr, E. A. and Riggs, D. S.: Protein-bound iodine in extra-thyroidal tissues. In preparation.
24. Chaikoff, I. L. and Taurog, A.: Studies on the formation of organically-bound iodine compounds in the thyroid gland and their appearance in plasma as shown by the use of radioactive iodine. Ann. N. Y. Acad., Sci., **50**: 377–402, 1949.
25. Chaney, A. L.: Improvements in determination of iodine in blood. Indust. & Engin. Chem., Anal. Ed., **12**: 179–181, 1940.
26. Chapman, E. M. and Riggs, D. S.: Unpublished observations.
27. Charipper, H. A. and Gordon, A. S.: The biology of antithyroid agents. Vitamins and Hormones, **5**: 273–316, 1947.
28. Childs, D. S., Jr., Keating, F. R., Jr., Rall, J. E., Williams, M. M. D. and Power, M. H.: The effect of varying quantities of inorganic iodide (carrier) on the urinary excretion and thyroidal accumulation of radioiodine in exophthalmic goiter. J. Clin. Investigation, **29**: 726–738, 1950.
29. Clark, D. E., Moe, R. H. and Adams, E. E.: The rate of conversion of administered inorganic radioactive iodine into protein-bound iodine of plasma as an aid in the evaluation of thyroid function. Surgery, **26**: 331–340, 1949.
30. Cohn, B. N. E.: Absorption of compound solution of iodine from the gastrointestinal tract with special reference to the absorption of free iodine. Arch. Int. Med., **49**: 950–956, 1932.
31. Connor, A. C., Swenson, R. E., Park, C. W., Gangloff, E. C., Lieberman, R. and Curtis, G. M.: The determination of the blood iodine. Surgery, **25**: 510–517, 1949.
32. Cope, O.: Diseases of the thyroid gland. New England J. Med., **246**: 368–373, 408–417, 451–457, 1952.
33. Cortell, R. and Rawson, R. W.: Effect of thyroxin on response of thyroid gland to thyrotropic hormone. Endocrinology, **35**: 488–498, 1944.
34. Currens, J. H., Reid, E. A. S., MacLachlan, E. A. and Simeone, F. A.: Metabolic effects of the rice diet in the treatment of hypertension. New England J. Med., **245**: 354–359, 1951.
35. Curtis, G. M., Puppel, I. D., Cole, V. V. and Matthews, S. B.: The normal urinary iodine of man. J. Lab. & Clin. Med., **22**: 1014–1025, 1937.
36. Danowski, T. S., Gow, R. C., Mateer, F. M., Everhart, W. C., Johnston, S. Y. and Greenman, J. H.: Increases in serum thyroxine during uncomplicated pregnancy. Proc. Soc. Exper. Biol. & Med., **74**: 323–326, 1950.
37. Danowski, T. S., Huff, S. J., Wirth, P. M., Hill, G. C., Green, M. H. and Fetterman, G. H.: Protein-bound iodine in serum of rats receiving iodide or iodine with or without goitrogen. J. Clin. Endocrinol., **11**: 1254–1258, 1951.
38. Danowski, T. S., Johnston, S. Y. and Greenman, J. H.: Alterations in serum iodine fractions induced by the administration of inorganic iodide in massive dosage. J. Clin. Endocrinol., **10**: 519–531, 1950.
39. Danowski, T. S., Mateer, F., Weigand, F. A., Peters, J. H. and Greenman, J. H.: Serum iodine fractions in subjects receiving potassium iodide in small dosage. J. Clin. Endocrinol., **10**: 532–539, 1950.
40. Davison, R. A. and Curtis, G. M.: Acetone fractionation of blood and urinary iodine. Proc. Soc. Exper. Biol. & Med., **41**: 637–639, 1939.
41. Dempsey, E. W.: Fluorescent and histochemical reactions in the rat thyroid gland at different states of physiological activity. Endocrinology, **34**: 27–38, 1944.
42. DeRobertis, E.: Cytological and cytochemical bases of thyroid function. Ann. New York Acad. Sc., **50**: 317–335, 1949.
43. Elmer, A. W and Scheps, M.: La thyroxine est-elle éliminée par les reins chez l' homme normal et chez les basedowiens? Compt. rend. Soc. de biol. Paris, **115**: 968–970, 1934.
44. Ershoff, B. H. and Golub, O. J.: Effects of prolonged exposure to cold on the serum protein-bound iodine of the rat. Arch. Biochem., **30**: 202–206, 1951.
45. Evans, R. D.: Quantitative aspects of radiation carcinogenesis in humans. Unio Internat. contra Cancrum. Acta, **6**: 1229–1237, 1950.

46. Fredrickson, D. S., Forsham, P. H. and Thorn, G. W.: The effect of massive cortisone therapy on measurements of thyroid function. J. Clin. Endocrinol., **12**: 541–553, 1952.

47. Freedberg, A. S.: Personal communication.

48. Freedberg, A. S., Ureles, A., and Hertz, S.: The serum level of protein-bound radioactive iodine (I^{131}) in the diagnosis of hyperthyroidism. Proc. Soc. Exper. Biol. & Med., **70**: 679–682, 1949.

49. Goldberg, R. C. and Chaikoff, I. L.: Failure of the dinitrophenol-induced fall in plasma protein-bound iodine to stimulate augmented TSH production. Endocrinology, **49**: 613–616, 1951.

50. Goldberg, R. C. and Chaikoff, I. L.: Induction of thyroid cancer in the rat by radioactive iodine. Arch. Path., **53**: 22–28, 1952.

51. Goldsmith, E. D.: Phylogeny of the thyroid: descriptive and experimental. Ann. New York Acad. Sc., **50**: 283–316, 1949.

52. Goldsmith, R. E., Stanbury, J. B. and Brownell, G. L.: The effect of thyrotropin on the release of hormone from the human thyroid. J. Clin. Endocrinol., **11**: 1079–1094, 1951.

53. Gordon, A. H., Gross, J., O'Connor, D. and Pitt-Rivers, R.: Nature of the circulating thyroid hormone-plasma protein complex. Nature, **169**: 19–20, 1952.

54. Gottschalk, C. W. and Riggs, D. S.: Protein-bound iodine in the serum of soldiers and of eskimos in the Arctic. J. Clin. Endocrinol., **12**: 235–243, 1952.

55. Greenwald, I.: Is endemic goiter due to a lack of iodine? J. Clin. Endocrinol., **6**: 708–741, 1946.

56. Greenwald, I.: A note on Chatin and the hypothesis that endemic goiter is due to a lack of iodine. Science, **111**: 501–502, 1950.

57. Greenwald, I.: Endemic goiter: deficiency, intoxication, or infection? Tr. Am. A. Study Goiter, 369–377, 1950.

58. Greer, M. A.: Nutrition and goiter. Physiol. Rev., **30**: 513–548, 1950.

59. Greer, M. A.: The effect on endogenous thyroid activity of feeding desiccated thyroid to normal human subjects. New England J. Med., **244**: 385–390, 1951.

60. Greer, M. A.: The results of feeding desiccated thyroid to thyrotoxic subjects. J. Clin. Investigation, **30**: 644, 1951.

61. Greer, M. A. and Astwood, E. B.: The antithyroid effect of certain foods in man as determined with radioactive iodine. Endocrinology, **43**: 105–119, 1948.

62. Gross, J. and Leblond, C. P.: Distribution of a large dose of thyroxine labeled with radioiodine in the organs and tissues of the rat. J. Biol. Chem., **171**: 309–320, 1947.

63. Gross, J., and Leblond, C. P.: Metabolism of the thyroid hormone in the rat as shown by physiological doses of labeled thyroxine. J. Biol. Chem., **184**: 489–500, 1950.

64. Gross, J., Leblond, C. P., Franklin, A. E., and Quastel, J. H.: Presence of iodinated amino acids in unhydrolyzed thyroid and plasma. Science, **111**: 605–608, 1950.

65. Gross, J. and Pitt-Rivers, R.: Unidentified iodine compounds in human plasma. Lancet, **2**: 766–767, 1951.

66. Gross, J. and Pitt-Rivers, R.: The identification of 3:5:3′-l-triiodothyronine in human plasma. Lancet, **1**: 439–441, 1952.

67. Gross, J. and Pitt-Rivers, R.: Physiological activity of 3:5:3′-l-triiodothyronine. Lancet, **1**: 593–594, 1952.

68. Gutman, A. B., Benedict, E. M., Baxter, B. and Palmer, W. W.: The effect of administration of iodine on the total iodine, inorganic iodine, and thyroxine content of the pathological thyroid gland. J. Biol. Chem., **97**: 303–324, 1932.

69. Hamilton, J. G.: The rates of absorption of the radioactive isotopes of sodium, potassium, chlorine, bromine and iodine in normal human subjects. Am. J. Physiol., **124**: 667–678, 1938.

70. Harington, C. R.: Thyroxine: its biosynthesis and its immunochemistry. Proc. Roy. Soc. London, S. B., **132**: 223–238, 1944.

71. Harington, C. R.: Twenty-five years of research on the biochemistry of the thyroid gland. Endocrinology, **49**: 401–416, 1951.

72. Harsha, W. N.: Evaluation of the conversion of radioactive inorganic iodine to protein-bound iodine as a diagnostic aid in thyroid dysfunction. J. Clin. Endocrinol., **11**: 1524–1534, 1951.

73. Hartsock, C. L.: Iodized salt in the prevention of goiter: Is it a safe measure for general use? J. Am. M. A., **86**: 1334–1338, 1926.

74. Heinemann, M., Johnson, C. E. and Man, E. B.: Serum precipitable iodine concentrations during pregnancy. J. Clin. Investigation, **27**: 91–97, 1948.

75. Hertz, S., Roberts, A., Means, J. H. and Evans, R. D.: Radioactive iodine as an indicator in thyroid physiology. Iodine collection by normal and hyperplastic thyroids in rabbits. Am. J. Physiol., **128**: 565–576, 1940.

76. Hill, S. R., Reiss, R. S., Forsham, P. H., and Thorn, G. W.: The effect of adrenocorticotropin and cortisone on thyroid function: thyroid adrenocortical interrelationships. J. Clin. Endocrinol., **10**: 1375–1400, 1950.

77. Hum, R. F., Goldberg, R. C. and Chaikoff, I. L.: Effect of excess iodide upon anterior pituitary cytology of the completely thyroidectomized rat and its bearing on the question of extrathyroidal thyroxine synthesis. Endocrinology, **49**: 21–24, 1951.

78. Karel, L.: Gastric absorption. Physiol. Rev., **28**: 433–450, 1948.

79. Keating, F. R., Jr.: Personal communication.

80. Keating, F. R., Jr. and Albert, A.: The metabolism of iodine in man as disclosed with the use of radioiodine. Recent Progr. Hormone Research, **4**: 429–481, 1948.

81. Keating, F. R., Jr., Haines, S. F., Power, M. H. and Williams, M. M. D.: The radioiodine-accumulating function of the human thyroid gland as a diagnostic test in clinical medicine. J. Clin. Endocrinol., **10**: 1425–1464, 1950.

82. Keating, F. R., Jr., Wang, J. C., Luellen, T. J., Williams, M. M. D., Power, M. H. and McConahey, W. M.: The measurement of the iodine-accumulating function of the human thyroid gland. J. Clin. Investigation, **28**: 217-227, 1949.
83. Kelsey, M. P., Haines, S. F. and Keating, F. R., Jr.: Radioiodine in the study and treatment of thyroid disease: a review. J. Clin. Endocrinol., **9**: 171-210, 1949.
84. Kimball, O. P.: Induced hyperthyroidism. J. Am. M. A., **85**: 1709-1710, 1925.
85. King, T. W.: Observations on the thyroid gland. Guy's Hosp. Rep., **1**: 429-447, 1836.
86. Krayer, O.: Über Verteilung und Ausscheidung des Jodes nach Zufuhr von Schilddrüsenstoffen. Arch. f. exper. Path. u. Pharmakol., **128**: 116-125, 1928.
87. Kydd, D. M. and Man, E. B.: Precipitable iodine of serum (SPI) in disorders of the liver. J. Clin. Investigation, **30**: 874-878, 1951.
88. Laidlaw, J. C.: Nature of the circulating thyroid hormone. Nature, **164**: 927-928, 1949.
89. Larson, F. C., Coulson, D. M. and Albright, E. C.: A radiochemical method for the determination of micro quantities of thyroxine. J. Biol. Chem., **196**: 45-50, 1952.
90. Leblond, C. P. and Gross, J.: The mechanism of the secretion of thyroid hormone. J. Clin. Endocrinol., **9**: 149-157, 1949.
91. Lerman, J.: Iodine components of the blood. Circulating thyroglobulin in normal persons and in persons with thyroid disease. J. Clin. Investigation, **19**: 555-560, 1940.
92. Lerman, J. and Harington, C. R.: The physiological activity of tetrabromthyronine and tetrachlorthyronine. J. Clin. Endocrinol., **9**: 1099-1106, 1949.
93. Lerman, J. and Salter, W. T.: The maintenance requirements of myxedema patients. Clinical and chemical assay of commercial thyroid preparations. J. Pharmacol. & Exper. Therap., **50**: 298-309, 1934.
94. Levine, H., Remington, R. E. and Von Kolnitz, H. Studies on the relation of diet to goiter. II. The iodine requirement of the rat. J. Nutrition, **6**: 347-354, 1933.
95. Luellen, T. J., Keating, F. R., Jr., Williams, M. M. D., Berkson, J., Power, M. H. and McConahey, W M.: Relative measurement *in vivo* of accumulation of radioiodine by the human thyroid gland: comparison with radioactivity in peripheral tissues. J. Clin. Investigation, **28**: 207-216, 1949.
96. Man, E. B., Heinemann, M., Johnson, C. E., Leary, D. C. and Peters, J. P.: The precipitable iodine of serum in normal pregnancy and its relation to abortions. J. Clin. Investigation, **30**: 137-150, 1951.
97. Man, E. B., Kydd, D. M., and Peters, J. P.: Butanol-extractable iodine of serum. J. Clin. Investigation, **30**: 531-538, 1951.
98. Marine, D. and Kimball, O. P.: The prevention of simple goiter in man. J. Am. M. A., **77**: 1068-1070, 1921.
99. Mason, E. E. and Bloch, H. S.: Gastric secretion of iodide at low serum iodide levels. Proc. Soc. Exper. Biol. & Med., **73**: 488-491, 1950.
100. McArthur, J. W., Rawson, R. W., Means, J. H. and Cope, O.: Thyrotoxic crisis. An analysis of the thirty-six cases seen at the Massachusetts General Hospital during the past twenty-five years. J. Am. M. A., **134**: 868-874, 1947.
101. McClendon, J. F.: Iodine and the Incidence of Goiter. University of Minnesota Press, Minneapolis, 1939.
102. McClure, R. D.: Experiences with the thyroid problem in a Detroit clinic. Ann. Surg., **85**: 333-338, 1927.
103. McClure, R. D.: The incidence of operations for goiter in southern Michigan. Effect of iodized salt after twelve years' general use. J. Am. M. A., **109**: 782-785, 1937.
104. McConahey, W. M., Keating, F. R., Jr., and Power, M. H.: The behaviour of radioiodine in the blood. J. Clin. Investigation, **28**: 191-198, 1949.
105. McConahey, W. M., Keating, F. R., Jr. and Power, M. H.: An estimation of the renal and extrarenal clearance of radioiodide in man. J. Clin. Investigation, **30**: 778-780, 1951.
106. Means, J. H.: Evaluation of the several methods for treating Graves' disease available today. Ann. Int. Med., **25**: 403-411, 1946.
107. Means, J. H.: The thyroid and its diseases. 2nd Edition, J. B. Lippincott Co., Philadelphia, 1948.
108. Means, J. H.: The use of radioactive iodine in the diagnosis and treatment of thyroid diseases. Bull. New York Acad. Med., **24**: 273-286, 1948.
109. Means, J. H.: The thyroid hormone. Certain aspects of its elaboration in the body, the significance of its structure, and of its action on end organs. Bull. Johns Hopkins Hosp., **89**: 90-105, 1951.
110. Means, J. H.: The use of hormones, drugs, and radiations in the management of thyroid diseases. Bull. Johns Hopkins Hosp., **89**: 106-120, 1951.
111. Means, J. H., Lerman, J. and Salter, W. T.: The rôle of thyroxine iodine and total organic iodine in the calorigenic action of whole thyroid gland. J. Clin. Investigation, **12**: 683-688, 1933.
112. Miller, W. H., Anderson, G. W., Madison, R. K. and Salley, D. J.: Exchange reactions of diiodotyrosine. Science, **100**: 340-341, 1944.
113. Money, W. L., Kraintz, L., Fager, J., Kirschner, L. and Rawson, R. W.: The effects of various steroids on the collection of radioactive iodine by the thyroid gland of the rat. Endocrinology, **48**: 682-690, 1951.
114. Money, W. L. and Rawson, R. W.: The experimental production of thyroid tumors in the male rat. Tr. Am. A. Study Goiter, 171-178, 1947.
115. Morris, H. P., Dalton, A. J. and Green, C. D.: Malignant thyroid tumors occurring in the mouse after prolonged hormonal imbalance during the ingestion of thiouracil. J. Clin. Endocrinol., **11**: 1281-1295, 1951.
116. Morton, M. E., Chaikoff, I. L., Reinhardt, W. O. and Anderson, E.: Radioactive iodine as an indicator of the metabolism of iodine. VI. The formation of thyroxine and diiodotyrosine by the completely thyroidectomized animal. J. Biol. Chem., **147**: 757-769, 1943.

117. Morton, M. E., Perlman, I., Anderson, E. and Chaikoff, I. L.: Radioactive iodine as an indicator of the metabolism of iodine. V. The effects of hypophysectomy on the distribution of labelled thyroxine and diiodotyrosine in the thyroid gland and in the plasma. Endocrinology, **30**: 495–501, 1942.

118. Muller, H. J.: Radiation damage to the genetic material. Am. Scientist, **38**: 33–59, 399–425, 1950.

119. Myant, N. B., Corbett, B. D., Honour, A. J. and Pochin, E. E.: Distribution of radioiodide in man. Clin. Sc., **9**: 405–419, 1950.

120. Myant, N. B., Honour, A. J. and Pochin, E. E.: The estimation of radioiodine in the thyroid gland of living subjects. Clin. Sc., **8**: 135–144, 1949.

121. Myant, N. B. and Pochin, E. E.: The thyroid clearance rate of plasma iodine as a measure of thyroid activity Proc. Roy. Soc. Med., **42**: 959–961, 1949.

122. Myant, N. B. and Pochin, E. E.: The metabolism of radiothyroxine in man. Clin. Sc., **9**: 421–440, 1950.

123. Myant, N. B., Pochin, E. E. and Goldie, E. A. G.: The plasma iodide clearance rate of the human thyroid. Clin. Sc., **8**: 109–131, 1949.

124. Nelson, N., Palmes, E. D., Park, C. R., Weymouth, P. P. and Bean, W. B.: The absorption, excretion, and physiological effect of iodine in normal human subjects. J. Clin. Investigation, **26**: 301–310, 1947.

125. Nickson, J. J.: Dosimetric and protective considerations for radioactive iodine. J. Clin. Endocrinol., **8**: 721–731, 1948.

126. Oddie, T. H.: Analysis of radioiodine uptake and excretion curves. Brit. J. Radiol., **22**: 261–267, 1949.

127. Oddie, T. H. and Scott, R. K.: External measurement of radio-iodine in the thyroid gland. Brit. J. Radiol., **22**: 698–705, 1949.

128. Perlmutter, M. and Riggs, D. S.: Thyroid collection of radioactive iodide, and serum protein-bound iodine concentration in senescence, in hypothyroidism and in hypopituitarism. J. Clin. Endocrinol., **9**: 430–439, 1949.

129. Perlmutter, M., Weisenfeld, S., Slater, S. and Wallace, E. Z.: A study of the mechanism of the inhibition of the thyroid gland induced by ingestion of thyroid substance. J. Clin. Endocrinol., **12**: 208–227, 1952.

130. Perry, W. F.: The action of cortisone and A.C.T.H. on thyroid function. Endocrinology, **49**: 284–288, 1951.

131. Peters, J. P. and Man, E. B.: The relation of albumin to precipitable iodine of serum. J. Clin. Investigation, **27**: 397–405, 1948.

132. Peters, J. P., Man, E. B. and Heinemann, M.: Pregnancy and the thyroid gland. Yale J. Biol. & Med., **20**: 449–463, 1948.

133. Pitt-Rivers, R.: Mode of action of antithyroid compounds. Physiol. Rev., **30**: 194–205, 1950.

134. Pitt-Rivers, R. and Lerman, J.: The physiological activity of the optically active isomers of thyroxine. J. Endocrinol., **5**: 223–228, 1948.

135. Plummer, H. S.: Exophthalmic goiter in Olmsted County, Minnesota. Tr. A. Am. Physicians, **46**: 171–172, 1931.

136. Plummer, H. S. and Boothby, W. M.: Specific dynamic action of thyroxine. Am. J. Physiol., **55**: 295–296, 1921.

137. Pochin, E. E.: Investigation of thyroid function and disease with radioactive iodine. Lancet, **2**: 41–46, 84–91, 1950.

138. Purves, H. D., and Griesbach, W. E.: The significance of the Gomori staining of the basophils of the rat pituitary. Endocrinology, **49**: 652–662, 1951.

139. Quimby, E. H., Werner, S. C. and Schmidt, C.: Influence of age, sex, and season upon radioiodine uptake by the human thyroid. Proc. Soc. Exper. Biol. & Med., **75**: 537–540, 1950.

140. Raben, M. S.: The paradoxical effects of thiocyanate and of thyrotropin on the organic binding of iodine by the thyroid in the presence of large amounts of iodide. Endocrinology, **45**: 296–304, 1949.

141. Raben, M. S. and Astwood, E. B.: The use of radioiodine in physiological and clinical studies on the thyroid gland. J. Clin. Investigation, **28**: 1347–1366, 1949.

142. Rall, J. E.: Iodine compounds in the blood and urine of man. J. Clin. Endocrinol., **10**: 996–1006, 1950.

143. Rall, J. E., Keating, F. R., Jr., Power, M. H., and Bennett, W. A.: The distribution of radioiodine in a patient with metastatic adenocarcinoma of the thyroid. J. Clin. Endocrinol., **9**: 1379–1391, 1949.

144. Rall, J. E., Power, M. H. and Albert, A.: Distribution of radioiodine in erythrocytes and plasma of man. Proc. Soc. Exper. Biol. & Med., **74**: 460–461, 1950.

145. Rand, C. G., Riggs, D. S. and Talbot, N. B.: The influence of environmental temperature on the metabolism of the thyroid hormone in the rat. In press.

146. Randall, R. V. and Albert, A.: The effect of hypophysectomy on the uptake of radioactive iodine by the thyroid gland of the rat. Endocrinology, **48**: 327–333, 1951.

147. Randall, R. V., Lorenz, N. and Albert, A.: The effect of hypophysectomy on the biologic decay of thyroidal iodine. Endocrinology, **48**: 339–340, 1951.

148. Rappport, R. L. and Curtis, G. M.: The clinical significance of the blood iodine: a review. J. Clin. Endocrinol., **10**: 735–790, 1950.

149. Rawson, R. W.: The use of radioactive iodine in studying the pathologic physiology of thyroid disease. J. Clin. Investigation, **28**: 1330–1346, 1949.

150. Rawson, R. W. and McArthur, J. W.: Radioiodine. its use as a tool in the study of thyroid physiology. J. Clin. Endocrinol., **7**: 235–263, 1947.

151. Rawson, R. W. and Money, W. L.: Physiologic reactions of the thyroid stimulating hormone. Recent Progr. Hormone Research, **4**: 397–428, 1948.

152. Rawson, R. W., Rall, J. E. and Peacock, W.: Limitations and indications in the treatment of cancer of the thyroid with radioactive iodine. J. Clin. Endocrinol., **11**: 1128–1142, 1951.

153. Recant, L. and Riggs, D. S.: Thyroid function in nephrosis. In press.

154. Riggs, D. S.: Serum protein-bound iodine as a diagnostic aid. Tr. Am. A. Study Goiter, 137–144, 1947.

155. Riggs, D. S.: Elevation of serum protein-bound iodine after large doses of radioactive iodine. Federation Proc., **7**: 251, 1948.
156. Riggs, D. S.: Renal clearance of iodide in the dog. Federation Proc., **8**: 328, 1949.
157. Riggs, D. S., Lavietes, P. H. and Man, E. B.: Investigations on the nature of blood iodine. J. Biol. Chem., **143**: 363-372, 1942.
158. Riggs, D. S., Man, E. B. and Winkler, A. W.: Serum iodine of euthyroid subjects treated with desiccated thyroid. J. Clin. Investigation, **24**: 722-731, 1945.
159. Riggs, D. S. and Stanbury, J. B.: Unpublished observations.
160. Rosenberg, I. N.: Column chromatography of thyroid gland hydrolysates. J. Clin. Endocrinol., **11**: 1063-1078, 1951.
161. Rosenberg, I. N.: The nature of the circulating thyroid hormone in Graves' disease. J. Clin. Investigation., **30**: 1-10, 1951.
162. Salter, W. T.: The Endocrine Function of Iodine. Harvard University Press, Cambridge, Mass., 1940.
163. Salter, W. T.: The metabolic circuit of the thyroid hormone. Ann. New York Acad. Sc., **50**: 358-376, 1949.
164. Salter, W. T.: A background for biological studies with radioiodine. Science, **109**: 453-456, 1949.
165. Salter, W. T., Cortell, R. E. and McKay, E. A.: Goitrogenic agents and thyroidal iodine: their pharmacodynamic interplay upon thyroid function. J. Pharmacol. & Exper. Therap., **85**: 310-323, 1945.
166. Salter, W. T., De Visscher, M. and McAdams, G.: Thyroxine metabolic turnover rate (T.M.R.). Tr. A. Am. Physicians, **64**: 364-371, 1951.
167. Salter, W. T., De Visscher, M., McAdams, G. B. and Rosenblum, I.: Changes in blood iodine fractions and radioactivity under therapy. J. Clin. Endocrinol., **11**: 1512-1523, 1951.
168. Salter, W. T. and Johnston, MacA. W.: Tracing the thyroid hormone in peripheral tissues. J. Clin. Endocrinol., **8**: 911-933, 1948.
169. Salter, W. T., Karandikar, G. and Block, P.: Organic and inorganic iodine: their reciprocal metabolic fates. J. Clin. Endocrinol., **9**: 1080-1098, 1949.
170. Sandell, E. B. and Kolthoff, I. M.: Microdetermination of iodine by a catalytic method. Microchim. Acta, **1**: 9-25, 1937.
171. Seed, L., Jaffé, B. and Baumeister, C.: The tracer dose of radioactive iodine in the diagnosis of thyroid disease. J. Clin. Endocrinol., **11**: 1143-1156, 1949.
172. Sheline, G. E. and Clark, D. E.: Index of thyroid function: estimation by rate of organic binding of I^{131}. J. Lab. & Clin. Med., **36**: 450-455, 1950.
173. Sheline, G. E., Moore, M. C., Kappas, A. and Clark, D. E.: A correlation between the serum protein-bound iodine and the radioiodine conversion ratio in various thyroid states. J. Clin. Endocrinol., **11**: 91-97, 1951.
174. Silver, S.: The nature of the blood iodine and its determination. J. Lab. & Clin. Med., **28**: 329-335, 1942.
175. Skanse, B.: Radioactive iodine in the diagnosis of thyroid disease. Acta med. Scandinav. Suppl. **235**, 1949.
176. Smith, H. W.: The Kidney. Structure and function in health and disease. Oxford University Press, New York, 1951.
177. Soley, M. H. and Foreman, N.: Radioiodine therapy in Graves' disease. A review. J. Clin. Investigation, **28**: 1367-1374, 1949.
178. Spector, H., Mitchell, H. H. and Hamilton, T. S.: The effect of environmental temperature and potassium iodide supplementation on the excretion of iodine by normal human subjects. J. Biol. Chem., **161**: 137-143, 1945.
179. Stanbury, J. B., Brownell, G. L., Riggs, D. S., Perinetti, H., del Castillo, E. and Itoiz, J.: The iodine deficient human thyroid gland. A preliminary report. J. Clin. Endocrinol., **12**: 191-207, 1952.
180. Stanbury, J. B. and Hedge, A. N.: A study of a family of goitrous cretins. J. Clin. Endocrinol., **10**: 1471-1484, 1950.
181. Stanley, M. M.: The direct estimation of the rate of thyroid hormone formation in man. The effect of the iodide ion on thyroid iodine utilization. J. Clin. Endocrinol., **9**: 941-954, 1949.
182. Stanley, M. M. and Astwood, E. B.: Determination of the relative activities of antithyroid compounds in man using radioactive iodine. Endocrinology, **41**: 66-84, 1947.
183. Stanley, M. M. and Astwood, E. B.: The accumulation of radioactive iodide by the thyroid gland in normal and thyrotoxic subjects and the effect of thiocyanate on its discharge. Endocrinology, **42**: 107-123, 1948.
184. Stanley, M. M. and Astwood, E. B.: Response of thyroid gland in normal human subjects to administration of thyrotropin as shown by studies with I^{131}. Endocrinology, **44**: 49-60, 1949.
185. Taurog, A., Briggs, F. N. and Chaikoff, I. L.: I^{131}-labeled l-thyroxine. II. Nature of the excretion product in bile. J. Biol. Chem., **194**: 655-668, 1952.
186. Taurog, A. and Chaikoff, I. L.: On the determination of plasma iodine. J. Biol. Chem., **163**: 313-322, 1946.
187. Taurog, A. and Chaikoff, I. L.: The nature of the circulating thyroid hormone. J. Biol. Chem., **176**: 639-656, 1948.
188. Taurog, A., Chaikoff, I. L. and Tong, W.: The nature of plasma iodine as revealed by filter paper partition chromatography. J. Biol. Chem., **184**: 99-104, 1950.
189. Taurog, A., Tong, W. and Chaikoff, I. L.: The monoiodotyrosine content of the thyroid gland. J. Biol. Chem. **184**: 83-97, 1950.
190. Thompson, W. O., Cohen, A. C., Thompson, P. K., Thorp, E. G. and Brailey, A. G.: The range of effective iodine dosage in exophthalmic goiter. III. The effect on basal metabolism of the daily administration of one-quarter drop of compound solution of iodine and of slightly smaller doses, with a summary of results to date. Arch. Int. Med., **45**: 430-455, 1930.

191. Thompson, W. O., McLellan, L. L., Thompson, P. K. and Dickie, L. F. N.: The rates of utilization of thyroxine and of desiccated thyroid in man: the relation between the iodine in desiccated thyroid and in thyroxine. J. Clin. Investigation, **12**: 235-246, 1933.

192. Thompson, W. O., Thompson, P. K. and Cohen, A. C.: The range of effective iodine dosage in exophthalmic goiter. IV. The effect on basal metabolism of the daily administration of about 0.75 mgm. of iodine. Arch. Int. Med., **49**: 199-214, 1932.

193. Tong, W., Taurog, A. and Chaikoff, I. L.: Nature of plasma iodine following destruction of the rat thyroid with I^{131}. J. Biol. Chem., **195**: 407-413, 1952.

194. Trunnell, J. B., Duffy, B. J., Jr., Godwin, J. T., Peacock, W., Kirschner, L. and Hill, R.: The distribution of radioactive iodine in human tissues: necropsy study in nine patients. J. Clin. Endocrinol., **10**: 1007-1021, 1950.

195. Tucker, R. G. and Keys, A.: Concentration of serum protein-bound iodine in normal men. J. Clin. Investigation, **30**: 869-873, 1951.

196. Uotila, U. U.: On the role of the pituitary stalk in the regulation of the anterior pituitary, with special reference to the thyrotropic hormone. Endocrinology, **25**: 605-614, 1939.

197. Vanderlaan, W. P. and Greer, M. A.: Some effects of the hypophysis on iodine metabolism by the thyroid gland of the rat. Endocrinology, **47**: 36-47, 1950.

198. Vanderlaan, J. E. and Vanderlaan, W. P.: The iodide concentrating mechanism of the rat thyroid and its inhibition by thiocyanate. Endocrinology, **40**: 403-416, 1947.

199. Wallace, G. B. and Brodie, B. B.: The distribution of administered iodide and thiocyanate in comparison with chloride, and their relation to body fluids. J. Pharmacol. & Exper. Therap., **61**: 397-411, 1937.

200. Werner, S. C., Quimby, E. H. and Schmidt, C.: The use of tracer doses of radioactive iodine, I^{131}, in the study of normal and disordered thyroid function in man. J. Clin. Endocrinol., **9**: 342-354, 1949.

201. Winkler, A. W., Riggs, D. S. and Man, E. B.: Serum iodine in hypothyroidism before and during thyroid therapy. J. Clin. Investigation, **24**: 733-741, 1945.

202. Wolff, J. and Chaikoff, I. L.: Plasma inorganic iodide as a homeostatic regulator of thyroid function. J. Biol. Chem., **174**: 555-564, 1948.

203. Wolff, J., Chaikoff, I. L., Goldberg, R. C. and Meier, J. R.: The temporary nature of the inhibitory action of excess iodide on organic iodine synthesis in the normal thyroid. Endocrinology, **45**: 504-513, 1949.

204. Wolff, J., Chaikoff, I. L., Taurog, A. and Rubin, L.: The disturbance in iodine metabolism produced by thiocyanate. The mechanism of its goitrogenic action with radioactive iodine as an indicator. Endocrinology, **39**: 140-148, 1946.

205. Wolff, J., Rubin, L. and Chaikoff, I. L.: The influence of 2,4-dinitrophenol on plasma protein-bound iodine. J. Pharmacol. & Exper. Therap., **98**: 45-48, 1950.

206. Wood, J. L. and Kingsland, N.: Labeled sulfur uptake by thyroids of rats with low plasma thiocyanate levels. J. Biol. Chem., **185**: 833-837, 1950.

207. Wood, J. L. and Williams, E. F.: The metabolism of thiocyanate in the rat and its inhibition by propylthiouracil. J. Biol. Chem., **177**: 59-67, 1949.

208. Wyngaarden, J. B., Stanbury, J. B. and DuToit, C. H.: On the mechanism of iodide accumulation by the thyroid gland. J. Clin. Endocrinol., **11**: 1259-1272, 1951.

209. Wyngaarden, J. B., Wright, B. M. and Ways, P.: The interfering action of certain anions with thyroid iodide accumulation and retention in the propylthiouracil-treated rat. In press.

210. Zilversmit, D. B., Entenman, C. and Fishler, M. C.: On the calculation of "turnover time" and "turnover rate" from experiments involving the use of labeling agents. J. Gen. Physiol., **26**: 325-331, 1943

THE DRAMA BEHIND YOUR DAILY WORK

Medical Milestones

By **HENRY J. L. MARRIOTT, M.D.**
Assistant Professor of Medicine, University of Maryland School of Medicine
With a Foreword by **Sir Alexander Fleming**

Factual, authentic account, written by a member of the medical profession, of the truly phenomenal advances in medical treatment of the last few years—completely devoid of the sensationalism too often found in the lay press, yet fascinating reading for both doctor and patient.

CONTENTS

- BEWARE THE IMPOSTOR
- THE MOLD ERA—Early Antibiotics
- FIRST AND STILL FOREMOST—Penicillin
- THREAT TO TUBERCULOSIS—Streptomycin
- A TRUCE WITH TYPHUS AND TYPHOID—Chloromycetin
- THE WIDEST RANGE—Aureomycin
- BACTERIA AGAINST BACTERIA—Bacitracin and Polymyxin
- MEDICAL SHRAPNEL—New Treatments for Anemia
- HORMONES AND CANCER—Chemical Warfare on Malignancy
- SPOILED SWEET CLOVER—Less of That Clotting
- GOITERS SLOWED DOWN—The Anti-Thyroid Drugs
- BLUE BABIES TO PINK—And Other Developments in Heart Surgery
- ANIMAL FACTORS IN THE BLOOD—The Rh Story
- FROM POISON GAS TO MEDICINE—British Anti-Lewisite
- "IF ANYONE TOUCHES A LEPER"—Drugs and Attitudes
- HOPE FOR THE RHEUMATIC—Cortisone and ACTH
- "MILLION-MURDERING DEATH"—Cornering Malaria
- ONCE BITING, TWICE SHY—Insects and DDT
- MAL DE MER—Dramamine
- ALL THAT GLITTERS

Etymological notes, scattered plentifully throughout the text, give you much useful and entertaining information about the origin and development of many common medical terms.

305 pp. *6 figs.* *$3.50*

THE WILLIAMS & WILKINS COMPANY

Mt. Royal and Guilford Aves. Baltimore 2, Maryland

Krantz & Carr—

Pharmacologic Principles of Medical Practice

second edition

Clinical Applications of the Action of Drugs

By JOHN C. KRANTZ, Jr., Ph.D.
Professor of Pharmacology, School of Medicine, University of Maryland;
Secretary, General Committee of Revision, U. S. Pharmacopeia;
and C. Jelleff Carr, Ph.D.
Associate Professor of Pharmacology, School of Medicine, University of Maryland

Second edition extensively revised in response to the many critical and constructive suggestions made by the users of the first edition.

In the second edition the central purpose remains unchanged: to present the pharmacodynamic and pharmacotherapeutic actions of drugs as you use them in the treatment and cure of disease.

New chapters: "The Chemotherapy of Tuberculosis", "The Chemotherapy of Rickettsial Diseases", "Anti-motion Sickness Drugs", "Treatment of the Arthritides; the Adrenal Corticotropic Hormone and Cortisone".

New illustrations and tables, some former figures redrawn, recent references added.

Text brought abreast with the 14th Revision of the U. S. Pharmacopeia, the 9th Edition of the National Formulary, and New and Non-Official Remedies, 1950.

1152 pp. 95 figs. 24 portraits $10.00

THE WILLIAMS & WILKINS COMPANY
Mt. Royal and Guilford Aves. Baltimore 2, Maryland